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DERMATOLOGICAL SOCIETY

Oral Treatment of Fungus Infections with Griseofulvin

An International Symposium

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The St. John's Hospital Dermatological Society

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TRANSACTIONS OF THE

ST. JOHN'S HOSPITAL DERMATOLOGICAL SOCIETY

(Incorporating the London Dermatological Society)

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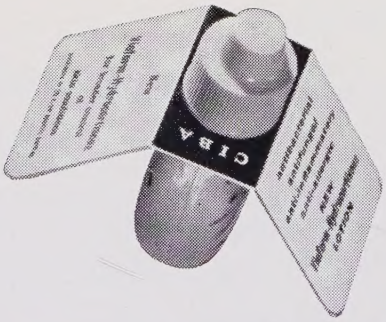

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THE SYMPOSIUM

At a time when leadership in dermatology has apparently passed from Europe to the United States, it is encouraging to find that contributions of the utmost value and importance for dermatologists continue to appear from European countries. Two notable examples are the acantholysis concept in pemphigus and the recognition of keratoacanthoma; both of these were accepted rather slowly, and perhaps hesitantly, by American dermatologists. The opening of the antibiotic era with penicillin by the late Sir Alexander Fleming was a proud achievement for British medicine, and now the discovery of griseofulvin is an event of which we in Britain may feel equally proud. One may, however, experience the same disturbance of conscience as when one recalls the long intermission between Fleming's original discovery and its application to human disease. For it has taken almost twenty years to obtain the benefits for our patients of the isolation of griseofulvin by Oxford, Raistrick, and Simonart in Oxford. Nystatin was apparently also left on the shelf for many years, because it did not have the particular anti-bacterial action which was being sought at the time. There is no doubt that efforts are being made in laboratories all over the world to avoid a repetition of this story. The screening programme for new antibiotic substances and new chemicals in pharmaceutical firms is vast compared to what was used twenty years ago. There is little doubt that the lessons of past events in the antibiotic field have been learnt, and if griseofulvin was discovered today there would be little or no lag period before its effects were appreciated and utilised for the benefit of mankind.

The story of the more recent development of griseofulvin is an interesting one. Although its antifungal activity was known more than a decade ago, its early application was virtually limited to the botanical sphere in which Brian and his colleagues of Imperial Chemical Industries made the first contributions. He showed the drug to be effective against the common dermatophytes, and suggested its use as a topical fungicide. Suitable preparations were put out on trial to a few dermatologists, but none of them were enthusiastic about the results, since these were no better than the currently used fungicidal creams and lotions. Some, at least, of those dermatologists may be regretting that they did not pay closer attention to the sheaf of technical data and information supplied to them before their trials. About the same time, however, Martin (also at Imperial Chemical Industries) had thought about the possibility of systemic treatment of dermatophytoses, and saw the significance of Brian's finding that griseofulvin could be taken up by the roots of plants and transported to the sites of fungal infections on the leaves. In April 1956 he effectively prevented experimental infections in guinea-pigs by the drug when given orally or parenterally. In retrospect, it is perhaps unfortunate, but understandable, that publication of these experiments was held up pending the necessary toxicity studies before the drug could be used in man. No praise can be too high for the meticulous and rigorous tests to which griseofulvin was submitted by the toxicology research team at I.C.I. It passed them all with only one exception—the remarkable colchicine-like effect when given in large doses intravenously. This sole fact and its implications was enough to stop the drug being used at once for benign infections. None will envy the medical advisors in pharmaceutical firms who have the responsibility for making such decisions.

Although Professor P. C. Koller was later to show that the arrest of chromosome division in the metaphase was but a transient, and apparently unimportant, effect, cautious trials in man were started after Gentles' very conclusive experiments in Glasgow had independently (and unknowingly) confirmed Martin's findings. A small number of patients began to receive griseofulvin in London and in Vienna before the publication of Gentles' paper on 16th August, 1958; following it a whirlwind blew up. Almost by chance Dr. Hildick-Smith of Johnson and Johnson Inc., New Jersey, read the paper in *Nature,* appreciated its significance, and communicated with Harvey Blank

in Miami, Florida. It so happened that Blank had a most unusual patient under his care—a man with chronic rheumatoid arthritis on long term steroid therapy and a widespread Trichophyton rubrum infection with multiple granulomas and extensive abscess formation of which the fungus was the only causative agent found. This man had received every possible form of medication without benefit, and he seemed likely to die. Blank felt there was nothing to be lost and prescribed a daily dose of 5 gms. of griseofulvin, which was supplied by air from Glaxo Laboratories Ltd. Within a week the improvement was astonishing, and the patient never looked back—except for cutaneous relapses when therapy was interrupted. Throughout the autumn of 1958 David Williams and his colleagues at King's College Hospital, Professor Gustav Riehl in Vienna, and several other investigators in various parts of the world, in addition to Harvey Blank accumulated evidence that griseofulvin was a remarkably safe, and a remarkably effective, drug for dermatophytoses. But it was Blank's presentation at the American Academy of Dermatology Meeting in Chicago in December 1958, which really publicised to the American continent the magnitude of this essentially British discovery. It virtually "stopped the show" in Chicago. The scientific programme was altered to include it and repeat performances were given. It was reported in the Wall Street Journal, and no doubt many stockbrokers without ringworm infections have benefited from griseofulvin.

All credit is due to Glaxo Laboratories and Imperial Chemical Industries who expanded their production and supplied the drug in large quantities to numerous investigators all over the world before it was finally marketed in April 1959. It is perhaps a sign of the times that less than six months after it was available for sale in the United States, an international symposium took place in Miami on October 26th and 27th, 1959, and now we have had a similar symposium at the Royal Society of Medicine in London on May 13th and 14th, 1960. On reading the proceedings of these two symposia, it is easy to see that the experience of most of the contributors is, inevitably, limited; but meetings on a national or even international scale at this stage do serve a very valuable purpose. They bring together a group of investigators for discussion of achievements to date as well as a reappraisal of the planned research ahead.

It is clear that there still exist very great differences in the organisation of dermatology in Britain and the United States. According to Professor D. M. Pillsbury, "Prior to World War II, it could safely be said that there was not a single investigative center in the United States in which broad-scale studies with the skin as the chief organ of reference were being carried out with adequate physical facilities, and with personnel trained in the various disciplines which must be brought to bear. . . . Following the War, the Rockefeller Foundation evidenced some interest in extending the scope and caliber of dermatologic investigation, and gave some support to a few institutions. . . . Even greater impetus was obtained through the National Advisory Health Council of the National Institutes of Health, when it declared dermatologic investigation to be a "neglected" area. . . . A more recent phase of this progress has occurred in the establishment of research training grants in dermatology. . . . I can report that in several Departments of Dermatology in this country, the facilities and personnel for detailed broadly basic studies are most impressive. Without specifying places, I should say there are three such centers on the west coast, possibly three in the mid-west, and three in the east."

Who will declare dermatologic investigation a "neglected" area in Britain? There is scarcely at present a single Department of Dermatology in this country equipped with either the personnel or the physical facilities to compare with the American centers. No department has a spectrophotofluorometer or an electron microscope. The problems posed by griseofulvin have served to highlight the deficiencies of our departments. The Symposium has indicated for us many fruitful avenues for research; can we find the human and material resources to follow them? The griseofulvin story has stressed the

need for yet closer collaboration between pharmaceutical firms, research workers, and practising clinicians, and a greater mingling between the medical staff of hospitals and pharmaceutical medical officers.

This symposium was a notable event in the history of St. John's Hospital Dermatological Society. All who attended it will feel grateful to the President and his organising committee, as well as to Dr. H. M. Walker of Glaxo Laboratories Limited and to Dr. A. S. R. Stewart of Imperial Chemical Industries Limited, Pharmaceutical Division, who gave much help and encouragement, and whose firms provided generous financial grants to make the symposium possible.

CONTROL OF FUNGAL DISEASES OF PLANTS WITH GRISEOFULVIN

P. W. Brian

As long ago as 1951 it was shown to be possible to protect plants against fungal attack by systemic treatment with griseofulvin (Brian, Wright, Stubbs & Way, 1951). Some of the factors found to influence its activity in plants may be relevant to its use in control of animal dermatophytes. A few of these are briefly discussed below; the subject has been treated in greater detail recently elsewhere (Brian, 1960).

In Vitro Antifungal Activity of Griseofulvin

The antifungal activity of griseofulvin was first observed in germination tests using spores of the plant pathogen *Botrytis allii* (Brian, Hemming & McGowan, 1945; Brian, Curtis & Hemming, 1946). Hyphae produced in its presence are distorted in various ways, depending on the concentration used. These distortions may take the form of excessive branching, shortening of hyphal 'internodes' between successive branches, swollen hyphal tips, intercalary swellings, knot-like twisting of hyphae or helical spiralling. These morphogenetic disturbances are accompanied by a greatly reduced growth rate, indeed growth may virtually cease. Nevertheless the activity of griseofulvin is essentially fungistatic rather than fungicidal. There is some evidence (Brian, 1960) that sensitivity to griseofulvin is dependent on cell-wall composition, and that the morphogenetic disturbances which it induces are in some way due to interference with normal synthesis of cell-wall constituents, and their orderly incorporation in new cell-walls. However, little is known about cell-wall formation in fungi, and this aspect of griseofulvin activity deserves more study.

The effect of griseofulvin on dermatophytes is essentially similar to its effect on *B. allii;* other fungi may be much more resistant, especially those with cellulose cell-walls (Brian, 1949).

The net effect of this interference with the growth of individual hyphae is a marked reduction in the rate of radial spread of colonies on agar surfaces, and in short-term experiments this inhibition of growth persists unchanged; and other measures of metabolic activity, such as a respiration or nutrient uptake, are reduced *pro rata.*

In longer term experiments, in which growth is measured by dry weight of mycelium, we have found with *B. allii* growing on the surface of liquid media, that even in the presence of relatively high concentrations of griseofulvin growth eventually proceeds (Brian, 1960). Thus in such experiments the effect of griseofulvin is mainly to increase the 'latent period' before rapid growth commences, the length of the latent period being related to the dose of griseofulvin. In other words adaptation occurs. One can envisage several methods by which such an adaptation may develop, for instance a change in permeability to the drug, enhanced production of a metabolic antagonist to the drug, development of some metabolic pathway insensitive to the drug, or enhanced capacity for metabolically detoxicating it. I think the last of these possibilities has been eliminated, but all the others remain as possible mechanisms of adaptation. The possibility that similar adaptation to griseofulvin may occur in dermatophytes cannot be ignored.

Relationship Between Chemical Structure and Activity

Griseofulvin has been shown (Grove, MacMillan, Mulholland & Rogers, 1952) to have the structure shown in Fig. 1, where $R = OCH_3$. Dechlorogriseofulvin and bromogriseofulvin, where the chlorine atom is replaced by hydrogen or bromine atoms respectively, are less active against *B. allii in vitro* than griseofulvin. On the other hand

the *n*-butyl and *n*-propyl homologues of griseofulvin (where the substituent *R* is OBu^n or OPr^n respectively) are of much greater activity, causing helical spiralling of *B. allii* hyphae at less than one-twentieth of the minimal effective concentration of griseofulvin (Crowdy, Grove & McCloskey, 1959).

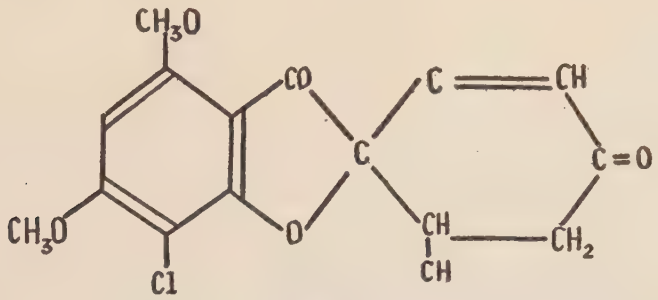

Fig. 1

Systemic Distribution in Plants

Nearly all the more detailed work on factors influencing systemic distribution of griseofulvin in plants has been concerned with conditions where the drug is presented to the roots (Stokes, 1954; Crowdy, Gardner, Grove & Pramer, 1955; Crowdy, Grove, Hemming & Robinson, 1956). In these conditions, passage from the root surface to the leaves involves at least two distinct processes. Passage from the root surface to the vascular system of the root is a complex process, rapid, independent of transpiration, and probably linked with respiration. Translocation upwards into the shoot through the vascular system is passive, and the amount translocated is linearly related to the amount of water transpired. This means that in the plant, treated by this route, the dose of griseofulvin reaching the leaves cannot be controlled unless we have a high degree of control of the environment, which is not normally possible in practice.

It is interesting to find that the *n*-butyl and *n*-propyl homologues of griseofulvin mentioned earlier, though more active *in vitro,* are quite useless for systemic control of plant disease. In fact, they enter the root but never leave the root tissues, scarcely any being passed on to the vascular system. Crowdy, Grove & McCloskey (1959) have attributed the failure of those compounds to their low water solubility, coupled with a high oil: water partition coefficient, which results in the compounds being bound in a lipoid phase of the root. Thus a systemic 'fungicide' for use in plants must have critical physical properties in addition to intrinsic antifungal activity. This is almost certainly true also of systemic antifungal agents for use in animals.

A further point worth making here is that most of the griseofulvin reaches the leaves unchanged and probably is antifungally effective as such in the leaves (Aytoun, 1956; Brian, 1960). However, some is broken down; it has been shown that the half-life of griseofulvin in bean tissue is of the order of 4 days. It is, of course, highly probable that the rate of destruction of griseofulvin is different in different tissues, and in different species of plant. This also is a consideration which may be relevant to animal systems.

Griseofulvin in Agriculture and Horticultural Practice

A fungicide must usually in practice be sprayed on the foliage of plants it is wished to protect; root treatment is either impractical, or too expensive, or both. Used in this way griseofulvin has been shown to give a degree of control of many plant diseases, particularly some of the mildews, which are in some ways analogous to fungal skin infections of animals. However, it must be admitted that in most cases griseofulvin is no better than established fungicides with little or no systemic effect. This I believe

is largely because its systemic properties have not been exploited, a consequence of the mode of application. Though some griseofulvin movement within the leaf can be detected when griseofulvin is applied locally on the leaf surface (Davis & Rothrock, 1956), such movement is slight. This could be expected, as the studies on root-treated plants have shown that bulk movement of the drug is mainly dependent on the transpiration stream. It is probable too that penetration of the griseofulvin through leaf surfaces is less than through root surfaces, but quantitative data are lacking.

I would not wish to be too pessimistic about the future of griseofulvin in practical plant protection. It is possible that better formulation or more suitable methods of application may give more promising results. There may be some diseases where griseofulvin is appreciably better than existing fungicides; the brown-rot of apples caused by *Sclerotinia fructigena* (Byrde, 1959) may be such a case. However, I have no doubt that its real future is in human and veterinary medicine.

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THE MANUFACTURE OF GRISEOFULVIN

A. H. Campbell

It is not intended here to describe in detail the manufacture of griseofulvin but merely to outline the process, dwelling particularly on the mycological aspects.

In 1939 Oxford, Raistrick and Simonart[1] isolated, described and named griseofulvin; they also set out their method of making it. For this a simple synthetic medium (modified Czapek-Dox), distributed as 350 ml. portions into litre flasks, was sown with spores of *Penicillium griseofulvin* and incubated for 65-68 days until, that is, the initial 8% glucose content had been virtually exhausted. Thereupon the mycelial felt that had meanwhile grown on the surface of the medium was harvested, washed, dried and finely ground. After preliminary treatment to remove oil, the dried felt was extracted with ether for 4 days, to yield a crude solid amounting in weight to about 10% of the felt. Further purification gave crystalline griseofulvin, about 50 g. from 3,000 g. of dried felt. This translated into a more convenient index, based on the volume of culture medium used, makes the yield nearly 200 mgm. of griseofulvin per litre.

Ten years later, Brian, Curtis and Hemming[2], using 11-14 days surface cultures of *Penicillium janczewskii*, showed that, by extracting both felt and filtrate, the total yield could be raised to about 400 mgm./litre. Thus, for a surface-culture process a possible doubling of the original yield could be forecast, in spite of a much reduced incubation time.

In 1952, we decided that the unusual activity shown by griseofulvin against a wide range of fungi justified a full technical appraisal of its likely prospects as an antifungal substance in a variety of applications. Since one possible use lay in agriculture, it was desirable to have some experience of large-scale production by submerged culture, that is, by the present technique for manufacturing antibiotics and some other products, which has replaced the original surface method for nearly 15 years. Thus an adequate supply of the antibiotic would be assured and, at the same time, we should gain the insight into costs of production required for any economic assessment.

For griseofulvin, as well as for the other antibiotics made by fermentation, the crucial matter is the yield (or "titre") obtained in the culture medium per unit incubation time. Clearly the aim must be the highest possible titre in the shortest possible time. Only when the process is established is it timely to consider the use of cheaper culture nutrients and other savings that may contribute to a reduction in the cost of the product.

For the fermentation studies, a prime requirement was rapid and accurate assay methods for determining the griseofulvin content of culture fluids. In 1946, Brian[3] had described a tube dilution assay based on the various morphological responses of *Botrytis allii* to a range of griseofulvin concentrations. This was unfortunately by its nature neither rapid nor accurate enough for our purposes. Similarly, the accurancy of plate assays, long the basic tool in fermentation studies, was affected by the aerial growth habits of test fungi, since direct contact between a hypha and the griseofulvin solution is necessary for elicitation of the inhibitory effect.

In this situation physical methods of assay became necessary. Griseofulvin can now be determined either spectrophotometrically, with correction for irrelevant absorption[4], [5], or polarimetrically, by virtue of the compound's high dextro-rotation. An isotope dilution assay based on chlorine^{36}-labelled griseofulvin is also available.[6] More recently a spectrofluorometric method has been developed for the assay of griseofulvin in tissues.[7] Although this has wider applications, it is unlikely to replace the well-tried spectrophotometric assay, which has been the mainstay of process development and other work.

The first problem encountered was the choice of strain for making griseofulvin. From earlier work it was already clear that at least three different species of *Penicillium* pro-

duced griseofulvin under surface culture conditions, though not necessarily in deep culture. A survey was made of many known *Penicillium* species and before long seven were selected as giving useful yields of griseofulvin by submerged culture. One strain of *Penicillium patulum* was found to be particularly suitable, in that it gave high yields under various deep culture conditions and, moreover, showed no substantial tendency to produce dechlorogriseofulvin under our conditions: this compound is considered valueless as having markedly less antifungal activity.

By use of the selected strain medium development was pushed forward rapidly. Rhodes, Crosse and Ferguson[8] described in 1956 various successful media in which they preferred to use complex organic materials as sources of nitrogen. They instanced corn-steep liquor, milk products and cotton-seed, oat and soya-bean meals, used singly or as mixtures. The preferred sources of carbon and energy were given as lactose, glucose, sucrose or starch. Although it was possible to produce griseofulvin over a wide range of values for the available nitrogen content of the medium, they found that the nitrogen level markedly influences the yield and that, for satisfactory yields, it must be controlled at rather lower levels than might be expected.

About a year earlier Aytoun and McWilliam[9] had described the production and selection of high-yielding mutants from the parent strain of *Penicillium patalum*. They showed that, if a griseofulvin-producing strain was grown on or in a nutrient medium in which the greater part of the nutrient sulphur source was present as radioactive isotope sulphur^{35}, the mutations that occurred included a few new strains having enhanced yields. Though the new mutation technique, in common with the many others known, gave rise to a variety of mutants differing in their power to make griseofulvin, these investigations showed not only that the new method yielded a greater percentage of viable mutant spores, but also that the percentage of mutants giving enhanced yields was surprisingly increased. This is thought to be because the radioactive sulphur, on being assimilated by the cell, is incorporated into its genic system by normal growth processes. Sulphur^{35} has a conveniently short half-life of about 87 days, with about 1% of the sulphur atoms undergoing radioactive decay daily. Upon decay, sulphur^{35} is transmuted into chlorine^{35}, that is, the chlorine isotope of mass number 35; and thus any group containing it is likely to lose its original function. If the grouping so affected were significant for the function of the gene, this alteration should result in mutation.

The method originally adopted was to seed the mould over the surface of a radioactive sulphur-containing medium and incubate until sporulation occurred. The spores were then harvested and plated out at suitable concentrations to give well-separated colonies. From each colony a master culture was prepared and subsequently used to seed small-scale test-fermentations for determining the griseofulvin yield. The yield potential of many thousands of colonies have to be examined, since the vast majority of the isolated strains reproduce the parent characteristic unchanged. Such work is laborious and expensive. It is not uncommon for a major mutation programme to call for the yield-testing of 1,000 miniature deep fermentations per month. Empirical as it may be, the selection and testing of induced mutants is one of the most successful and important parts of the antibiotic fermentation industry today.

It is clear that if techniques could be devised for cutting out the isolation and testing of colonies showing precisely the same characteristics as the parent strain, the operation would become much more efficient. This can be done by selecting for specific biochemical mutants that are recognised by their dependence or otherwise upon a particular amino acid or other essential nutrient in the culture medium. However, Aytoun (personal communication) has developed a method depending upon the demonstration of morphological changes in a colony grown upon the radioactive sulphur-containing substrate. A morphological change under these conditions is evidently an overt symptom of physiological change and therefore identifies a mutant strain. Aytoun's technique is to place a few spores in the center of a petri dish containing the radioactive substrate and incubate for a lengthy period to produce a single giant colony. In the course of its growth, a number of sectors appear, differing from the parent in colour or growth

habit. By selecting from these sectors there is a good assurance that only mutants are being handled. By the use of these mutant techniques, a number of strains of greatly enhanced griseofulvin productivity were discovered. As shown by Aytoun and McWilliam[9], when such a mutant strain was used with the improved media described by Rhodes, Crosse and Ferguson[8], yields of over 3,000 mgm./litre were obtained in 14 days.

The next step is to move from the laboratory scale, from the miniature fermenter with a working volume of about one gallon, to production tanks of 10 or 15 thousand gallon capacity. The change in magnitude brings many new problems; however, by proceeding through intermediate sized fermenters the laboratory process is gradually modified to achieve highest yields in the production tanks. At the end of the fermentation the mycelial felt is harvested. Provided this is done before autolysis begins, over 90% of the griseofulvin remains in the felt, and the filtrate may either be discarded or separately extracted. The felt, either wet or after drying, is then extracted with a suitable organic solvent, the extract being clarified and concentrated to produce crude griseofulvin of about 80% purity. Subsequent purification to "pharmaceutical grade" involves removing colour and recrystallisation to eliminate impurities, to give a final product of not less than 95% purity. This white crystalline solid, after passing stringent analytical and pharmacological standards, is then made into tablets.

In concluding this brief account of the mycological aspects of griseofulvin manufacture, reference may be made to a point found puzzling by many. Since griseofulvin causes typical morphological effects, such as curling, in the hyphae of *Penicillium*, how is it that moulds of this species become resistant to the inhibitory action of griseofulvin? In the dermatophyte *Microsporum canis*, for example, continued growth in the presence of low concentrations of griseofulvin brings a succession of morphological changes leading tubules, haematopoietic marrow, and experimental animal tumours. The results of tests favourable conditions in the fermenter, the typical curling effect is clearly seen, but growth is not significantly affected even at high concentrations. A similar effect is seen in *Fusarium* species, which do not produce griseofulvin at all. It must be concluded that the mode of action of griseofulvin is complex, varying considerably among the different fungus groups. There is a need for much more fundamental work on this matter, for from a better understanding might well come developments that would both enhance and extend the antifungal action of the antibiotic.

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EFFECTS OF GRISEOFULVIN ON EXPERIMENTAL INFECTIONS (I)

J. C. Gentles

The investigation of ringworm therapy by the oral route was undertaken because of the failure of a number of substances to influence the course of ringworm infections when applied topically, although all of them were active against dermatophytes *in vitro*. Since griseofulvin was known to have a low mammalian toxicity and since dermatophytes were sensitive to very low concentrations it was chosen for study first of all.

Griseofulvin, in aqueous suspension, was given daily by mouth at a level of 200 mg./kg. to guinea-pigs infected with *Trichophyton mentagrophytes*. Treatment was begun on the day of infection and the treated animals did not develop ringworm although all the control animals became infected. Oral griseofulvin given daily at a level of 75-100 mg./kg. starting on the fourth day after infection was also completely effective.

Further, more detailed studies were carried out using *Microsporum canis* as the infective agent. In these investigations treatment was withheld until the tenth day after infection and the course of the disease in both treated and control animals was followed by means of Wood's light and by examination of skin sections, as well as clinically and by microscopy and culture. The effect of griseofulvin, given daily in tablet form at a level of 60 mg./kg. was clearly evident within 4 days of commencing treatment when control animals, receiving blank tablets, had highly inflammatory lesions, whilst the lesions on the treated animals had regressed. Skin sections showed only half the hair follicles to be infected after 4 treatments, and after 8 treatments the infection was almost cleared from the follicles. In control animals almost every follicle was heavily invaded by the fungus.

The fluorescence under Wood's light had cleared from the base of infected hairs about a week after the first treatment, and microscopic examination of epilated hairs confirmed that the non-fluorescent parts of the hairs were not infected. The uninfected portion corresponded in length to approximately the growth of hair expected during the treatment period and in certain hairs malformation of hyphal tips, reminiscent of the effect of griseofulvin on susceptible fungi, was noted at the junction of the infected and uninfected portions. The hair keratin formed during treatment with oral griseofulvin was clearly resistant to invasion by the dermatophyte and it seemed likely that this resistance was a result of the incorporation of griseofulvin in the keratin.

In order to obtain further information on this probable mode of action efforts were made to recover griseofulvin from guinea-pig hair which had grown during a treatment period of approximately 3 weeks. Between 5 and 6 μgm. of a substance with griseofulvin-like activity was obtained from 1 gm. of hair (Gentles, Barnes and Fantes, 1959). It was assumed that the substance was griseofulvin and since about half of it was obtained by washing the hair in cold water it was considered that this portion may have been deposited on the outside of the hair in skin secretions. The remainder, which was extracted by boiling methanol, was probably incorporated in the hair keratin. Further investigations (Gentles and Barnes, 1960) in which use was made of a spectrophotofluorometric assay (Bedford, Child & Tomich, 1959) capable of identifying as well as determining the concentration of griseofulvin, confirmed that the substance extracted from hair of treated guinea-pigs was unchanged griseofulvin. These additional studies also produced evidence in support of the view that some of the griseofulvin is excreted through the skin and some is deposited within the hair keratin.

Efforts were made to find the minimum effective dose and the frequency and duration of treatment required for a complete cure. Surprisingly enough, it is now clear that there is remarkable agreement between the dosage schedule required for guinea-pigs and that

which is effective in humans. For guinea-pigs 15 mg./kg. daily is close to, if not the actual minimum effective dose and complete clearance of infection is obtained if treatment is continued until the lesion heals. Ten daily treatments of 15 mg./kg. was completely effective whereas 8 treatments allowed reinfection with *M. canis* to occur. On the other hand 4 treatments at a level of 30 mg./kg., given at intervals of 48 hours, cleared the infection completely. The explanation for this is not quite clear but it is probably related to the survival-time of the minimum effective blood-level and to the rate of keratin production.

Griseofulvin is completely effective against *T. mentagrophytes, M. canis* and *M. gypseum* ringworm in guinea-pigs and very recently I have had an opportunity of testing it against artificially induced infections with the rare, and recently described dermatophyte, *Microsporum nanum* (Fuentes, 1956) and the common soil fungus which is an occasional skin pathogen, *Keratinomyces ajelloi.* These also responded to treatment with oral griseofulvin given daily at a level of approximately 30 mg./kg. In the guinea-pig all these fungi cause highly inflammatory lesions and treatment was started when infections were well established but before the inflammation had fully developed. If treatment is delayed until the inflammatory response reaches its height quite unsatisfactory results are obtained. In my opinion this is the explanation for the reports by some clinicians of the failure of griseofulvin to influence the course of animal ringworm in humans caused by *Trichophyton verrucosum.* Clearly, although a kerion is caused by the dermatophyte, once it is formed its further development is quite independent of the fungus and will not be affected by griseofulvin which acts only on the dermatophyte. *T. verrucosum* is sensitive to griseofulvin *in vitro* and Lauder and O'Sullivan (1958) have shown the drug to be effective in treatment of cattle ringworm. Cochrane and Tullett (1959) found it effective for animal ringworm in humans.

Griseofulvin is fungistatic, and dermatophytes may remain in contact with it for long periods without loss of viability. It does not cause any permanent change in the morphology of dermatophyte cultures although, because isolations are usually made from griseofulvin-treated patients at frequent intervals during the period of treatment, and because dermatophytes are extremely variable, this has been suggested.

I have made several attempts to find resistant mutants of dermatophytes by exposing large numbers of spores to varying concentrations of griseofulvin *in vitro* and I have tested the sensitivity of many isolates from treated animals and patients. In neither case has there been any evidence of mutation to resistance. One can only hope that reports on this aspect will continue to be in the negative. It is perhaps of interest that I have found the strain of *K. ajelloi* which is pathogenic to be as sensitive to griseofulvin *in vitro* as *M. canis,* whereas soil isolates of *K. ajelloi* and another close relative of the dermatophytes, *Trichophyton terrestre,* which are non-pathogenic, are approximately 5 times more resistant. Does this mean by any chance, that if a dermatophyte develops a permanent increased resistance to griseofulvin its ability to parasitize keratin will be correspondingly reduced?

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EFFECTS OF GRISEOFULVIN ON EXPERIMENTAL INFECTIONS (II)

A. R. Martin

As most members of this audience are perhaps more familiar with the use of drugs than with their discovery, a brief discussion of the difficulties which face those of us who have to try to find new ones may not be out of place.

We must, of course, have some "model" of the conditions we hope to treat. This should be in the smallest possible laboratory animal, to economise in the amounts of experimental drugs used. It is often thought that it is essential for the experimental infection to mimic closely the clinical condition which is the objective of the work. This view was expressed by Sulzberger and Baer (1954) in the following words: "We know of no infection in animals which runs a sufficiently prolonged and accurately predictable course to make it a suitable biologic object for the comparative evaluation of external or systemic measures against the common superficial mycoses affecting the skin, hair and nails of man." Robinson and Robinson have expressed similar views as recently as 1959.

Acceptance of this view (which, in my opinion, is mistaken) may account for the very small number of reported attempts to find chemotherapeutic remedies for dermatophytoses. In general, it is sufficient to cause the parasite to produce some measurable change in the host. The nature and time-scale of this change need not simulate closely those of the natural infection in the natural host, for if the changes in the experimental disease can be convincingly influenced by a drug, that is as far as the laboratory worker can hope to go, or indeed, needs to go in the first instance. In the present condition it is very fortunate that this is so, for natural infections with dermatophytes are characteristically very slow in their development—a property which would make them very unattractive as experimental subjects. The experimental infections are, on the contrary, very acute and, although self-limiting, do allow chemotherapeutic effects to be demonstrated. After examining four other species of laboratory animal we decided to confine ourselves to the infection of guinea-pigs with *Trichophyton mentagrophytes*. This was produced by applying a suspension of spores of the organism to lightly scarified areas on the flanks of the animal. Under our conditions, this infection reached its peak in 9 days and then rapidly resolved spontaneously.

Granted, then, that one has a suitable experimental "model" infection, how does one decide with what new or old chemical compounds to attempt to treat it? In my experience, the choice of a speculative compound for trial against an experimental infection is governed by one or more of the following factors:

(a) It is chemically related to a compound already known to have the desired property.

(b) If sufficiently distorted on paper its formula can be made to resemble some known metabolite.

(c) It happens to be available.

(d) It is easy to make.

These may not appear to be very impressive qualifications, but in spite of the vast amount of information available about the metabolic intermediates and processes of many mammalian tissues, and of the bacterial, fungal, protozoal and other parasites which attack them, the only clinically useful drug whose constitution was predicted by study of information of this type is, as far as I know, para-aminosalicylic acid. In some instances the mode of action of a drug has been explained in terms of interference with

known metabolic processes, but this information has come after, not before, the discovery. As it happens, the recognition of the therapeutic value of griseofulvin was slightly more logical than the rather haphazard process I have just outlined.

When we started work on the chemotherapy of dermatophytoses in Imperial Chemical Industries laboratories in 1956 it was apparent that a new approach was needed. The very imperfect results obtained by the local application of the large number of fungistatic and fungicidal drugs available commercially indicated that fungi in the skin were not very accessible from the external surface, and it seemed probable that they might be accessible, by diffusion or otherwise, to blood-borne drugs.

In addition to all the publications of Dr. Brian and his collaborators on griseofulvin, we had available a number of his internal reports, in one of which the inhibitory effects of griseofulvin on dermatophytes *in vitro* were described. It is interesting to note that, in a report deated May 1954, Dr. Brian suggested that griseofulvin might well be used as a topical fungicide. However, it seemed to me that the ability of griseofulvin to penetrate the roots of plants, to be transported within the plant to the sites of fungal infection in the leaves, and there to control these infections was an obvious "lead" to a systemically-active fungicide in man or animals.

In experiments of this type we make it a rule, in our laboratories, the first time a new compound is examined, to weight everything in favour of the drug. In this first experiment, therefore, griseofulvin was given in a large dose, 100 mg./kg., by the intraperitoneal route to ensure absorption. Treatment was started at the same time as infection and was repeated daily for 8 days. The result of this experiment (carried out in April 1956) was extremely clear—the control animals showed well-developed lesions, whilst those treated with griseofulvin showed scarcely any abnormality of the skin. It is possibly the only occasion on record in which a useful new drug has been discovered in the first experiment of a series.

There seems little need to describe in detail the subsequent extensions of the original observation. It was readily shown that the drug was also effective orally and, by quantitative comparisons of the severity of the lesions in treated and control animals, that considerably smaller doses exerted some effect, although a less dramatic one. It was also possible to show some activity when griseofulvin was applied locally. These effects were neither as good as the best obtainable by oral administration nor so reliable.

The subsequent development of griseofulvin did not prove as straightforward as its discovery had been. Chronic toxicity studies showed that very large doses could be given orally to animals over several weeks without causing more than a slight depression of growth rate, but its administration intravenously, again in large doses, caused the very unusual cytotoxic changes which have been described by my colleagues Dr. Paget and Dr. Walpole (1958). These changes were judged to be of such serious import, should they occur in an exceptionally sensitive individual, that they caused us to be extremely cautious—perhaps excessively so—in recommending griseofulvin for the treatment of dermatophytoses. However, cautious trials controlled by close observation of haematological constants disclosed no seriously untoward result, and the place of griseofulvin in therapeutics is now being defined. In this instance we did not need to enlist the aid of our chemical colleagues to develop the "lead"—fortunately, for it poses many difficult chemical problems—but we do not imagine the last chapter in the treatment of dermatophytoses has been written, and we hope to find alternatives to griseofulvin.

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DISCUSSION

DR. BROSSI: As Dr. Campbell told us, some sorts of Penicillium do produce a deschlorogriseofulvin and, as Dr. Brian told us, the bromo-analogue of griseofulvin also can be produced. Is it possible to make other changes in the aromatic ring of griseofulvin, for instance, fluoro-griseofulvin by fermentation? What do you think of a total synthesis as a possible procedure?

DR. CAMPBELL: I think that would take me so deep into the chemistry that this would hardly be an appropriate place to deal with it. All I can say is that, as Dr. Brian indicated, there is really an infinity of compounds which could be made by working around the griseofulvin molecule, and we are in fact setting out to do a job of work on that. I am afraid that is really all I can say on it.

DR. LATOUCHE: I should like to ask a question which might apply equally to Dr. Campbell's or Dr. Brian's paper. Do they know of any relation between oxygen tension and the effect of griseofulvin on dermatophytes? I have found in some preliminary experiments with different concentrations of griseofulvin that the mycelium—this is in liquid culture — which grows near the surface is much more prolific and grows very much better than that which is nearer the bottom of the tube.

THE CHAIRMAN: Could either of you help on that point? Have you noticed that variations in oxygen tension or proximity to a supply of oxygen have affected your cultures?

DR. BRIAN: I think the key to this observation is a point that Dr. Campbell made earlier, that it is only those fungal hyphae which are in contact with griseofulvin which are affected. All the evidence shows that the griseofulvin is not translocated with the fungus. So if you get a colony of which some parts are aerial, other parts sub-aerial and other parts submerged, the effect would be greatest in those parts which are submerged, and least in those which are not in direct contact with griseofulvin.

THE CHAIRMAN: Would it make any difference in the actual oxygen available?

DR. BRIAN: I would not have thought that was the explanation, but I might be wrong.

THE CHAIRMAN: Near the cell wall?

DR. BRIAN: I think it is a question of whether the griseofulvin is in contact with the cell wall.

I might mention that normal butyl and normal propyl derivatives when compared with griseofulvin *in vitro* are much more active than griseofulvin, although systemically in parts they are useless. The reason is that if you apply them to the root they stay there and do not move at all. It can be suggested, and it is probably true, that because of their lower water solubility and higher fat solubility they tend to get held up in some hyphal phase in the root. So, to get systemic control, you have to have something that it is not only active *in vitro,* but something that is capable of translocation. We have seen this is some plants.

THE CHAIRMAN: Has anyone here any special experience or view on griseofulvin for fungus conditions associated with plants of any kind?

If there are no comments we will leave that for a moment. Would anyone care to discuss the points brought up in Dr. Gentles' experiments? Or has anyone done a similar experiment with different results?

DR. CALNAN: I would like to ask Dr. Gentles a question. He said that he took the infected hairs from the animals that were on griseofulvin; when he washed the griseofulvin out aqueously before the ethanol extraction, if he planted those hairs would the fungus still grow? I ask this because it has been suggested that the drug could be simply washed out and allow the fungus to continue to grow.

DR. GENTLES: We have tried growing dermatophytes on hair from guinea-pigs which were not treated with griseofulvin, and we find it is difficult to obtain the

growth on single hairs, but we can grow the dermatophytes on a mass of hair in a humid, warm atmosphere. We then tested hairs from guinea-pigs which had been treated with griseofulvin for a considerable period, and discovered that again we could obtain growth on the mass of hair in a moist atmosphere. The griseofulvin contained in the hair or upon it does not appear to be sufficient to inhibit the growth of the dermatophytes.

DR. WHITTLE: I was rather disappointed to hear that Dr. Gentles banished the successful treatment of *Trichophyton verrucosum* infections so readily. I was wondering if he could perhaps clarify this point a little. Was it only those cases which developed kerion that he was rather gloomy about? Or was it all types of *Trichophyton verrucosum* infection in man?

DR. GENTLES: There were certain articles which stated that griseofulvin for *Trichophyton verrucosum* infection was not particularly successful. There are other reports, of course, to the contrary, which illustrate that griseofulvin is very successful in the treatment of verrucosum infections in humans. I would like to make the point that probably the people who did not obtain success were treating infections at a very late stage when the kerions had fully developed.

PROF. VANBREUSEGHEM: I should like to comment on Dr. Gentles' observation of the *in vitro* penetration of hairs from animals fed griseofulvin, and their invasion by dermatophytes. I wonder if he has seen the truth about it. I do not think that I have seen it either, but I have observed occasionally that hairs from the same patients who had received griseofulvin were *in vitro* invaded by dermatophytes others were not. What I mean is that some hairs were invaded and others were not invaded. I have not tackled this question sufficiently, but tomorrow morning I will come back with the same point about the nails.

DR. MACKENZIE: I should like to ask Dr. Gentles if he could answer a simple question; that is, whether griseofulvin, when administered early in cases of ringworm, could possibly reduce inflammation by itself. Is there any evidence to support this, for instance the fact that the fungus could remain viable for long periods? In a severe inflammatory case of ringworm is it not true that the fungus infection would die off rather rapidly; it loses viability? If griseofulvin will reduce inflammation in the early stage of infection, has this been supported by producing a viable fungal material over a long period of time?

DR. GENTLES: The point I was trying to make was that if you treat the infection early enough the griseofulvin will affect the fungal growth and reduce the inflammation which results from the growth of the fungus. If you leave your treatment until the kerion has fully developed there is very little fungus present because the inflammation has killed it off. The griseofulvin, therefore, cannot produce the same effect on the lesion.

Someone may wish to make a statement about griseofulvin and its anti-inflammatory effect. I myself am rather doubtful that it has any effect other than a direct effect on the growth of the fungus.

The CHAIRMAN: That has widened the field a little on the anti-inflammatory aspect.

DR. CALNAN: May I ask Dr. Gentles if he related his responses to the development of allergic sensitivity to trichophytin? Most of the inflammatory effect, in many cases, is of an allergic nature, and the allergic sensitivity varies, certainly in man, between dfferent people; some patients develop it early and some patients develop it late. The degree of allergic sensitivity varies too, and it is very often the degree of allergic sensitivity that determines the natural history of the untreated case. I should like to know if Dr. Gentles has studied this point in his experimental infections.

The CHAIRMAN: That is debatable. The actual question has two sides to it. Would you care to answer that now, Dr. Gentles?

DR. GENTLES: I should like only to say that with our guinea pigs the inflammatory response is equivalent in all animals; I do not find any variation in the response.

DR. CHATTAWAY: Returning to the point Dr. Gentles made that the fungus grew quite well on hairs from treated guinea pigs *in vitro*, is Dr. Brian's point not relevant here, that there may not be contact between the griseofulvin and the fungus if it is

combined with the keratin; and the fungus growing on the surface of the hair may grow quite readily because there is no contact?

Further to the point of combination with keratin, Dr. Gentles said that he extracted the hair with methanol which might reasonably extract loosely-bound griseofulvin. Has he tried further chemical degradation of the hair to see if more firmly-bound griseofulvin may not be present in larger quantities?

DR. GENTLES: I agree with Dr. Chattaway that the growth of hair from treated animals is related to the question of whether or not the fungus was in direct contact with the griseofulvin. The main point there was that we were not able to obtain growth on the single hair of an untreated animal and, therefore, we could not make a more sensitive test.

So far as the extraction is concerned, it was carried out by Dr. Fantes and Dr. Barnes, and I do not know whether either is present today and could answer the question. So far as I know there was no attempt to degrade the hair further and obtain additional amounts of griseofulvin.

QUESTION: I think one of the remarkable things about griseofulvin clinically is surely the way in which it is able to be selectively taken from the circulatory system, and brought rather selectively into tissue undergoing keratinization. I would like to ask Dr. Gentles if he has any experimental evidence at all that griseofulvin is involved in the actual process of keratinization.

DR. GENTLES: The question is whether griseofulvin is connected with the process of keratinization. My view is that griseofulvin is present in the living cells which produce the keratin, and when the cells die off the griseofulvin persists in the keratin. I am not qualified to answer the question as to whether it has any other connexion with the process of keratinization.

The CHAIRMAN: Has anyone conducted any experiments or noticed anything which might indicate that griseofulvin has any effect on otherwise normal keratin, or on the normal nail in the case where there has been an infected nail?

DR. MARTIN: A question has been asked as to whether griseofulvin has any direct anti-inflammatory action. I think the answer is that experimentally it has. At very large doses we have shown in our laboratories that you can clearly depress the response reaction in normal guinea pigs; that is with doses of the order of 200 mg. per kg. We have examined a number of other quantitative measures if inflammation and failed to demonstrate any effect with griseofulvin upon them. Our impression is that the anti-inflammatory action, though probably present, is not of very great importance.

DR. BOLAM: I would like to say that several of us here feel that it has an anti-inflammatory action in the patient. I think Dr. Whittle and Dr. Mackenzie were hinting at that in their questions, and I have certainly observed this. You do not now have to give other antibiotics in inflammatory ringworm such as kerion; it fades away after taking griseofulvin.

DR. WHITTLE: I was merely saying, very tentatively, that I have noticed changes in the normal nails in griseofulvin-treated patients. I have no pictures of them here, although I have colour photographs of them at home; there are things happening in the nails. Whether it is due to griseofulvin or to something else I do not yet know, but I think the lunulae are altering in appearance; they seem to be extending further down the nail. I would not like to go further than that at the moment; I am collecting further evidence.

DR. CURRY: I would like to ask Dr. Brian about the effect of bringing griseofulvin into contact with the plant invaders and whether there was any difference in the mode of attack of the pathogen on the plant. Obviously it would make some difference if the pathogen were a root invader or a surface invader.

DR. BRIAN: Most of the experimental work has taken this form: — griseofulvin was applied to healthy plants through the roots, and the leaves of the plants were then artificially infected with a fungal pathogen. The griseofulvin treatment protected the

plants against fungal attack. Much less work has been done on root pathogens, and I don't think we have enough information to come to any general conclusions.

DR. BARLOW: Since a certain amount of the griseofulvin can be recovered from the hair by washing, I wonder if anybody knows how much can be recovered by washing an animal after a test dose, whether the total amount can be recovered.

DR. GENTLES: I am afraid I cannot answer that. I have done no work which has involved washing an animal.

The CHAIRMAN: In view of the expense it might be of considerable interest.

DR. SHAPERO: I would like to ask Dr. Martin whether, during his programme for anti-fungal agents, he finds any correlation between *in vivo* and *in vitro* results.

DR. MARTIN: No. I have found hundreds with *in vivo* action, but no others that I know of have a systemic action; there are very few that have.

DR. LATOUCHE: May I ask whether those who treat tinea capitis think that washing the hair frequently during griseofulvin treatment might mitigate the effect of griseofulvin on the hair, in view of the fact that water extracts griseofulvin from the hair? This is relative to the question that was answered a few minutes ago.

The CHAIRMAN: Has anyone any experience of a patient not doing so well because of over-washing?

DR. MEARA: I may be able to answer that in part. The children I am treating for tinea capitis all have a scalp shampoo every day and they seem to be doing quite well; it does not interfere with the treatment.

The CHAIRMAN: It took them no longer to get well?

DR. MEARA: I did not control the experiment, but it seems to have been quite normal. Results were achieved in the expected time, and they seem to have been the same as those obtained by everyone else.

DR. MARTIN: One would surely not expect washing to have much effect on tinea capitis, since the important growing part of the fungus is just above the bulb in the infected hair and is presumably not reached in the washing process. If the griseofulvin is there it will remain there, I should imagine.

PROF. VANBREUSEGHEM: I would like to say something about the influence of vascular factors on griseofulvin in experimental tests on mice. I have observed that if you give griseofulvin at the same time as you are giving something which dilates the vessels, initially the quantity of griseofulvin fixed by the nails is greater than it is in the animals which have received griseofulvin only. However, after a longer period the nails seem to contain less griseofulvin. It is possible to explain this by the fact that the vasodilating substance produces at the same time a diuretic action, so probably a good quantity of the griseofulvin given to the animal was eliminated in excess urine.

DR. THORNE: Has any work been done on combining griseofulvin systemically in animals or in plants, with either topical griseofulvin or another fungicide to see if you get any better action, or if you can cut down the amount of griseofulvin given systemically by so doing?

DR. WILLIAMS: For a long time, of course, we have been saying that there is no really satisfactory basis for thinking that local applications should not work in the treatment of the dermatophyte infections. We are beginning now—perhaps because of the failure of griseofulvin by mouth in a certain number of cases—to think again of local applications. Most people have tended to use griseofulvin alone, for the reason that the addition of local applications complicates the picture and makes it difficult; you add another variable to something which has enough variables anyway. However, recently, because of these failures, I have tried local griseofulvin for the treatment of a few dermatophyte infections on the feet and on the palms. I have used it in association with griseofulvin only in the treatment of cleft infections, and it makes no difference at all—they still do not get better.

PROF. RIEHL: I think it has a certain value to combine, especially in the case of toe-nail and finger-nail infections, because I think the natural desire we have is to try to get rid of those fungi that are not approachable by the blood stream. So we try to

eradicate the remaining part of the nail as quickly as possible by mechanical or chemical means. I think we have been able to show in a great number of cases that there is a reduced time of treatment if we use the combined method. I am not quite sure whether I am right, but that is the way we do it now. The main thing is to get the patient healed quickly because of the cost of griseofulvin, which is a very great difficulty in our country where the price is very high. So we try to save griseofulvin as much as we can, and we combine all sorts of therapeutic procedures, with the griseofulvin by mouth.

The CHAIRMAN: I will not ask anyone to elaborate on that particular angle now because we are going to discuss the clinical side of this problem later.

Does anyone wish to speak essentially on the last three papers?

DR. LATOUCHE: Kaplan and Ajello (1959) in the United States have carried out some trials with orally administered griseofulvin on cats, and have found that they achieved much more success if, in addition to the orally administered griseofulvin, they washed the cats with a substance called Captan. The relevant sections from their paper in the Journal of the American Veterinary Medical Association (1959.135.253) are as follows:

'To expedite the conversion to a culturally negative state, it was considered advisable to apply topical therapy. Accordingly, 9 cats were dipped in the Captan solution and 3 were shampooed with the napthalene soap. Specimens were then taken and cultured the same day and two weeks later. After one immersion in the dip, 4 cats (9, 10, 12, and 17) became culturally negative; 5, however, still yielded *M.canis* on culture. The three that were given the shampoo treatment were rendered free of infection. One cat (13) had not received topical therapy and still yielded *M.canis* after three months of griseofulvin treatment.'

(Re. griseofulvin) 'Its principal limitation, however, lies in the inability to regularly eradicate the infectious agent from the body surface within a reasonably brief period of time. To facilitate the achievement of this ultimate goal, supplemental therapy in the form of dips or shampoos might be advantageously employed.'

The CHAIRMAN: Before we conclude the discussion on these papers, is there anyone else who has something he wishes to bring out?

DR. JEREMIASSE: Could I ask Dr. Brian what strength griseofulvin he uses in treating lettuces to prevent damping off? Secondly, should you treat them just after planting out, or has it any lethal effect on them?

DR. BRIAN: In our early experimental work on *Botrytis* infection of lettuces we treated the roots of the seedlings with 10 p.p.m. griseofulvin and this protected the seedlings against leaf infection. I have no experience of practical protection of lettuce crops, but I imagine one might spray the plants, before an attack of *Botrytis* is expected, with a suspension of griseofulvin at about 100 p.p.m. Dr. Campbell will be able to answer this better than I can.

DR. CAMPBELL: If I can add to that, the use of griseofulvin in this country in the agricultural context is at the present time only applied to glass-house lettuces. Lettuce in January and February, as you have probably noticed, is rather an expensive item and, in order to be quite sure that they raise a clean crop, growers can afford to pay for griseofulvin. The form of use is three per cent dust—that is, three per cent griseofulvin in an inert powder—and that is dusted on the lettuces at the time when they anticipate there could be infection.

The CHAIRMAN: I suppose really one has to face the fact that if we are all eating a lot of griseofulvin-sprayed lettuce, it will not be long before the problem arises of what organisms are breeding inside us. I wonder what is going to happen to orthodox Hindus who are very restricted in their vegetarian diet, if they are coping with this problem.

GRISEOFULVIN IN NATURAL AND EXPERIMENTAL INFECTIONS IN CATS AND CHINCHILLAS

by
C. J. La Touche

PART I

ORAL GRISEOFULVIN TREATMENT OF CATS NATURALLY AND EXPERIMENTALLY INFECTED WITH MICROSPORUM CANIS (BODIN)

In cats or kittens naturally infected by *Microsporum canis*, recovery occurs spontaneously after a period which varies from 2 or 3 months to 6 months or longer (up to 3 years). Irrespective of age, cats or kittens thus infected may transmit their infection to Man, although this is more likely to be the case with infected kittens which are more fondled by children, rather than with infected adult cats.

The control of the reservoir of *M.canis* infection in cats is rendered difficult because of the occurrence of "carriers" amongst these pets, which are detected usually only after a human contact has become infected. Even in the case of cats presenting with obvious lesions, control is difficult because of incorrect diagnosis and because, owing to a natural tendency on their part to wander, spread of infection to other cats, dogs and humans is likely. Control can be achieved by eliminating infected cats and kittens discovered because of their having transmitted their infection to humans. This has been done in Leeds (La Touche, 1959) at least temporarily, no more cases of infection due to *M.canis* having been recorded for 1959 or thereafter up to date.

Treatment of ringworm infection in cats is most desirable, especially in catteries, but has hitherto been unrewarding because such antifungal preparations as have been provided so far for topical application have failed to exert their fungicidal effect because of their apparent inability to penetrate the hair follicle sufficiently and likewise the hair root in the hair follicle. The discovery by Gentles (1958) that griseofulvin could do this when administered orally to experimentally infected guinea-pigs suggested that the same result could be achieved in humans suffering from scalp ringworm and also in ringworm-infected animals such as cattle, cats, dogs, and other fur-bearing animals. This has proved to be the case as regards humans, as a wealth of literature already testifies, and in cattle as shown by Lauder & O'Sullivan (1958), and also in cats as shown by Kaplan and Ajello (1959).

It is clear, however, that a drug administered internally cannot exert its effect on fungal elements lying on the skin surface or on the hair unless it be actually excreted on to these surfaces. The successes achieved by clinicians in the treatment of scalp ringworm have been due to the combined effects of internal and external therapy (i.e. oral administration of griseofulvin, together with clipping and shampooing the hair or applying antifungal drugs to the surface of the scalp). This is the rational approach to therapy with griseofulvin. How far it can be applied in the control of widespread ringworm infection in animals remains to be seen. Owing to the high cost of this drug there have been few reports of this form of treatment in ringworm in cattle. Only one paper reporting treatment of cats with griseofulvin on a large scale has so far appeared, that by Kaplan and Ajello (1959). These authors claimed successful treatment with orally administered griseofulvin in a number of spontaneously infected cats of different breed and age. In 22 cats it was possible to effect clinical recovery, after varying periods when clipping was practised, as assessed by the use of Wood's light. It was found,

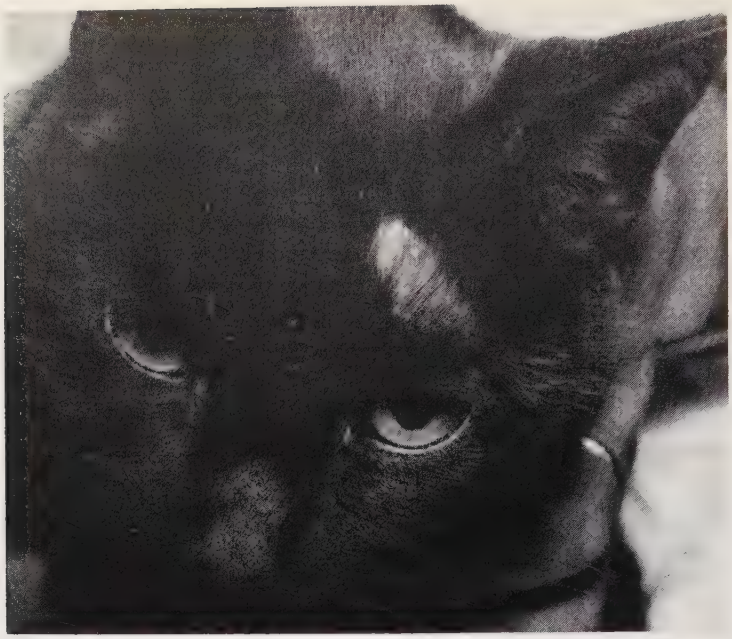

FIGURE 1

M.canis spontaneous infection. Lesion 10 days old, before start of treatment

however, that only 8 of these cats were rendered culturally negative through the use of griseofulvin alone. After 2 months' therapy with this drug "it was still possible to isolate *M.canis* from 13 of these cats." Success in the case of these 13 cats was achieved only after topical therapy with other drugs was superimposed on oral griseofulvin. These authors pointed out quite rightly, in reference to griseofulvin, that "its principal limitation, however, lies in the inability to regularly eradicate the infectious agent from the body surface within a reasonably brief period of time"; and they suggested that supplemental therapy in the form of dips or shampoos might be advantageously employed.

INVESTIGATIONS IN LEEDS

With the object of studying the effect of orally administered griseofulvin on the clinical course of *M.canis* ringworm in cats, some preliminary trials were carried out by the author—(a) with a naturally infected cat, and (b) with a cat in which infection by *M.canis* was induced.

MATERIAL AND METHODS

In October 1959, 4 out of 26 stock cats originating from Bradford in Yorkshire which were kept in the Leeds Medical School for various investigations, were found to be suffering from ringworm due to *M.canis* when they were examined as a result of one of the animal attendants becoming infected by this dermatophyte. Two of these cats were reserved for a therapeutic trial with griseofulvin. One of them, a black male weighing approximately 3.5 kgm. presented with a lesion on the left eyebrow (Fig. 1). This lesion was, at the initiation of the treatment, 3.5 cm. long by 1.0 cm. wide, was somewhat scaly over its whole surface, and included a small crust. Most of the hairs in

this lesion had been broken off, leaving stumps which fluoresced a green colour in Wood's light. The other cat was a black and white male weighing approximately 3 kgm. and presenting with a partly depilated lesion which extended over the whole of the bridge of the nose and included a number of Wood's light positive hairs. A small lesion with Wood's light positive hairs was present also on the right eyebrow.

Dosage

To the black cat with the lesion on the left eyebrow 3 tablets of griseofulvin (750 mgm.) representing approximately 214 mgm. per kgm. body weight, were administered orally in powder form per day for 6 days. Treatment with this drug was then subsequently maintained at a dosage of 500 mgm. per day for 45 successive days, making a total of 51 days, the total dose during this period amounting to 27 gm. of griseofulvin. The other cat served as control.

Results

Treated Cat

At 6 days from the start of the treatment most of the hairs in the lesion on the left eyebrow showed clearing of fluorescence up to about half way along the shaft.

At 7 days only the tips of most of the hairs in this lesion were fluorescent. A few Wood's light positive hairs were also observed on the lower lid of the left eye. The left eyebrow hairs were then shaved off, leaving a few fluorescent stumps.

At 19 days all fluorescence had disappeared from this lesion except for that exhibited by Wood's light positive hairs included in the encrusted portion.

At 26 days a few remaining Wood's light positive hairs were clipped from the left eyebrow, which thereafter remained clear.

At 36 days the Wood's light positive hairs on the lower lid of the left eye were still present.

In the meantime, at 15 days from the start of treatment, a hitherto unnoticed lesion was observed on the inferior aspect of the tail. In this lesion were some long Wood's light positive hairs exhibiting fluorescence along their whole extent, although some of the hairs were fluorescent at the tip only. Some Wood's light positive hairs were also present on the inferior aspect of the left hind leg.

At 23 days these hairs on the tail were clipped as much as possible, and also at 26 days.

At 38 days fluorescence was still showing in these tail hairs and once more they were clipped.

At 50 days Wood's light positive hairs were still present on the inferior aspect of the tail.

Treatment at this stage was suspended as apparently no progress was being made.

However, at 81 days it was decided to reinstitute treatment in a dosage of 3 tablets (750 mgm.) per day, and this was continued for 12 days; thereafter a maintenance dose of 375 mgm. per day was administered for 9 days.

At 107 days a scaly lesion including Wood's light positive hairs was discovered on the dorsum of the tail at the same level as that on the inferior aspect. Barium sulphide was applied to this to clear off the hairs included in the lesion, and treatment with 375 mgm. per day was resumed and continued for 10 days.

At 118 days, 2 Wood's light positive hairs were still present on the inferior aspect of the tail but were fluorescent at the tips only.

At 119 days, the tail appeared clinically clear.

At 144 days, 1 Wood's light positive hair was observed on the dorsum of the tail in this lesion as well as a crust.

At 146 days, 3 or 4 Wood's light positive hairs were present in the lesion on the upper side of the tail as well as a crust. This crust was removed and cultured, yielding

M.canis, as did also a scraping from the inferior aspect of the tail.

At 170 days, a scaly crust had reformed on the lesion on the dorsum of the tail. This contained mycelium and, on culture, again yielded *M.canis*.

Altogether, this cat has remained infected with *M.canis* for nearly 6 months since treatment with griseofulvin was initiated. At no time were topical antifungal drugs applied to the lesions. Three courses of griseofulvin were administered, viz. November 4 to December 24 (27 gm.); January 29 to February 20 (3.4 gm.) and February 25 to March 5 (3.75 gm.). The total amount of griseofulvin administered during this period was thus 34.1 gm. The *M.canis* strain used was sensitive *in vitro* to 5 mgm. per ml. of griseofulvin.

Control Cat

In the meantime the lesion on the nose of the control cat gradually cleared and the hair regrew. At 105 days (15 weeks) it was clinically and mycologically clear. During this time fluorescent hairs were observed on the right eyebrow and on the toes of the left and right hind paws, but had cleared from these sites within 30 days.

EXPERIMENTALLY INDUCED INFECTION WITH M.CANIS IN CATS

Experimental infection was induced in 3 cats with the same strain of *M.canis* as was originally isolated from the black and white cat which served as control in the first trial.

Material and Methods

A heavy suspension in sterile water was made from the surface growth (including mycelium and macroconidia) of a 2-week old Sabouraud glucose slope culture. The inner aspect of the ears of all 3 cats was then rubbed lightly with fine sandpaper. A drop of the *M.canis* culture suspension was then spread and rubbed into the slightly abraded surfaces and also a drop of this suspension was rubbed on to the bridge of the nose of each cat.

Results of Inoculations

At 14 days after inoculation green fluorescence appeared in some of the nose hairs of cat "A", a ginger and white male, and at 17 days a uniform band of fluorescence measuring 1.0 cm. x 0.5 cm. extended from eye to eye.

At 21 days cat "B", a tabby and white male "Angora" exhibited a few Wood's light positive hairs in the right ear, and in cat "C" 9 Wood's light positive hairs were present in the right ear.

At 25 days Wood's light positive hairs were present in both ears of cat "C".

At 41 days infection was well established in these sites in all 3 cats, and treatment of cat "B" was therefore commenced, the other 2 cats serving as controls.

At this date the clinical appearance of the lesions in these cats was as follows:

Cat "A"

The patch of Wood's light positive hairs on the nose measured 2 cm. x 2 cm. In the left ear a scarcely scaly erythematous area measured 1.5 cm. x 1.5 cm. and included a few Wood's light positive hairs. The inner aspect of the right ear exhibited a darkened patch but did not show any Wood's light positive hairs.

Cat "B"

A scaly Wood's light positive patch of hairs extended across the right ear from border to border and measured 2.5 cm. x 3.5 cm. The left ear did not reveal any features of note, and the nose appeared normal.

Cat "C"

In the right ear a scaly patch on the inner aspect including Wood's light positive

hairs measured 2.75 cm. x 1.75 cm. and extended from border to border. A few Wood's light positive hairs were also present along the margins of the ear on the dorsal aspect.

In the left ear a patch of Wood's light positive hairs accompanied by matted scaling measured 2.5 cm. x 2.5 cm.

The nose appeared normal.

TREATMENT

Griseofulvin was administered orally in powder form to cat "B" (which weighed approximately 3 kgm.) in a dosage of 375 mgm. per day (i.e. 125 mgm. body weight per day). This dosage was maintained for 38 days.

RESULTS: Right Ear

At 10 days fluorescence was less bright and there were signs of clearing at the bases of the hairs.

At 14 days fluorescence was present at the tips in a number of the hairs.

At 24 days there was much improvement, most of the hairs fluorescing at the tips only. As many as possible were clipped off, but this was difficult in the case of short hairs along the margins of the ear.

At 28 days, as fluorescence was still present, most of the hair was removed with barium sulphide.

At 38 days some short Wood's light positive hairs were still present along the margins of the ear. As many as was possible of these were removed by epilation. Treatment was suspended but reinstituted 2 days later for 4 days.

At 45 days no Wood's light positive hairs were observed and treatment was accordingly suspended.

At 56 days Wood's light positive hairs had reappeared along the margin of this ear.

At 65 days a scaly patch including Wood's light positive hairs was observed on the inner aspect of this ear.

At 90 days, as Wood's light positive hairs were still present in this patch, it was decided to re-institute treatment with 375 mgm. of griseofulvin per day as before.

At 101 days 6 Wood's light positive hairs were still present, but while 5 of them were fluorescent at the tips only, 1 was fluorescent uniformly. Accordingly, all these hairs were clipped, and an antifungal ointment, "Dicrophen," was applied. Two days later, as no fluorescence was observed, griseofulvin was withdrawn. So far 2 Wood's light positive hairs have been observed (at 106 days).

This cat has so far received a total of 19.875 gm. of griseofulvin.

Control Cats: *Cat "A"*: Nose

At 6 days a marked diminution in the extent of the fluorescent patch had taken place.

At 14 days this area had become reduced to a narrow band between the eyes.

At 24 days only 1 Wood's light positive hair remained.

At 30 days no Wood's light positive hairs were present.

Left Ear

There were 3 Wood's light positive hairs at 10 days.

At 24 days there were still 3 Wood's light positive hairs and a crust.

At 35 days 6 Wood's light positive hairs were present.

At 66 days this ear was clinically clear but a positive culture was obtained.

At 78 days this ear was still clinically clear but again a positive culture was obtained.

At 91 days this ear was both clinically and culturally clear.

In the meantime infection had spread to the right and left eyebrows, but lasted for only about 15 days. Likewise, infection had spread to the right hand paw where a patch about 1.5 cm. in diameter was present, but again this was of short duration.

Cat "C"

Left Ear

At 10 days hairs were still strongly Wood's light positive and showed no clearing, while some were present on the dorsum.

At 16 days hairs were still fluorescent along their whole extent.

At 24 days there were somewhat fewer Wood's light positive hairs but fluorescence was present along their whole extent.

At 28 days folliculitis was present. The hairs were removed with barium sulphide.

At 38 days the skin was somewhat crusty but only 1 Wood's light positive hair was present.

At 45 days no Wood's light positive hairs were present.

At 54 days this ear was almost clinically clear but culturally positive.

At 66 days it was culturally negative and likewise at 78 and 93 days.

Right Ear

At 10 days the hairs were still strongly Wood's light positive.

At 16 days hairs were fluorescent all along the shaft.

At 24 days hairs were still fluorescent all along the shaft.

At 28 days folliculitis was present and the hairs were removed with barium sulphide.

At 38 days a few faintly Wood's light positive hairs were present.

At 45 days 6 Wood's light positive hairs were present.

At 54 days no Wood's light positive hairs were present but a positive culture was obtained.

At 66 days cultures were again positive.

At 78 days no Wood's light positive hairs were present but culture was positive.

At 93 days no Wood's light positive hairs were present and culture was negative.

Conclusion

It is clear from these observations, that lesions in different sites do not resolve within the same period in naturally or experimentally infected cats. It is clear also that the response to treatment, at least in one naturally infected cat, is different in different lesions (c.f. eyebrow and tail).

These results indicate, as do the results of other authors, that some form of supplementary topical treatment is necessary to render the treatment with orally administered griseofulvin effective.

Summary

Preliminary trials with orally administered griseofulvin were conducted in a naturally infected and an experimentally infected cat (the etiologic agent being *M.canis*).

In the case of the naturally infected cat the initial dosage was 750 mgm. per day for 6 days and treatment was maintained for another 45 days on a dosage of 500 mgm. per day.

With clipping, a lesion on the left eyebrow of this cat was clinically clear at 26 days.

A lesion on the tail of this cat responded very slowly to this first course of treatment and two further courses.

A large lesion on the nose of a control cat was clinically and culturally clear at a much earlier date than the lesion on the tail of the treated cat.

In the case of the cat in which infection was induced, the initial dosage was 375 mgm. per day. This dosage was maintained for a total of 38 days.

There was definite response to this treatment within the first 14 days, but despite clipping and further treatment the lesion had not resolved at 100 days.

PART II

ORAL ADMINISTRATION OF GRISEOFULVIN TO CHINCHILLAS INFECTED WITH TRICHOPHYTON MENTAGROPHYTES

Two chinchillas, one an adult aged about 2 years and the other aged about 4 months, were submitted for investigation and treatment.

Adult Chinchilla

The adult chinchilla, on examination, presented with a depilated, erythematous, scaly and somewhat encrusted lesion which included the skin around the nose and to some extent that below the left eye (Fig. 2). A depilated, slightly scaly lesion was also present on the dorsum of the tail at its root (Fig. 3).

Young Chinchilla

The young chinchilla, on examination, presented with a depilated erythematous, scaly and somewhat encrusted lesion above the right eye reaching from the right side of the nose to the base of the right ear and extending to the midline of the forehead (Fig. 4). Another depilated, scaly and encrusted patch was present on the skin of the left shoulder measuring about 3 cm. in diameter.

Microscopical Examination and Culture

Both animals revealed mycelium in the scales and hair included in the lesions, as well as arthrospores on the hair. Culture on Sabouraud glucose medium containing penicillin, streptomycin and actidione yielded *T. mentagrophytes* var. *granulosum*.

Treatment

It was decided to treat the older chinchilla with griseofulvin and to reserve the younger one as a control. This older chinchilla weighed 379 gm. so it was thought that a dosage of 62.5 mgm. per day administered orally would be a suitable dose (i.e. 165 mg. per kgm. body weight per day). The griseofulvin was administered in powder form encased in a raisin (on the advice of the owner). This dosage was maintained for 20 days, i.e. a total of 1.25 gm. was administered.

Results: Treated Chinchilla

Improvement was gradual, but at 16 days hair was regrowing well in the lesion around the nose although some crusting remained.

At 20 days the lesion had almost completely resolved. Regrowth of hair on the tail lesion had also progressed, (but apparently not as quickly as that on the face). Microscopical examination of the hair and scales did not reveal any fungus in either of the lesions, but culture material taken from both lesions yielded *T. mentagrophytes*.

At 31 days these lesions had almost completely resolved, showing that progress had been maintained, but hairs obtained from both lesions showed on culture that the dermatophyte was still present on them in viable form.

Control Chinchilla

Resolution was much slower than in the case of the lesions in the treated chinchilla. At 20 days the lesions were still depilated, scaly and encrusted, and there was some

FIGURE 2a

(Above left). *T.mentagrophytes* infection of muzzle in chinchilla at start of treatment.

FIGURE 2b

(Above right). *T.mentagrophytes* infection of tail in chinchilla at start of treatment.

FIGURE 3

(Right). *T.mentagrophytes* infection in young chinchilla. Control — untreated at start of trial.

spread to the right ear. Microscopical examination of hairs and scales collected at 22 days, revealed mycelium in the hair and scales and arthrospores in the hair, and cultures were positive for *T. mentagrophytes.*

Conclusions

Orally administered griseofulvin appears to have a beneficial effect on lesions due to *T. mentagrophytes* in chinchillas, effecting a clinical cure in about 1 month. Nevertheless viable fungus can still be cultured in the presence of clinical cure. It is clear, therefore, that some form of topical application is required to dispose of potentially infective material present on treated and clinically cured animals. Assessment of clearance from infective material can be conducted only by culture.

Acknowledgement

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VETERINARY EXPERIENCE WITH GRISEOFULVIN

Olga Uvarov

Dermatophyte infections are important both for man and his domestic animals, but the incidence differs with species. Clinical ringworm in cattle is widespread (Ainsworth & Austwick, 1955), and in rural areas forms an important reservoir of infection to man (Gentles & O'Sullivan, 1957), but the incidence of the infection is low in dogs and cats. In cats the infection is often not noticed (La Touche, 1952; Fuentes, Bosch and Bondet, 1954), and at times is detected only when people in contact develop the infection. Small animals are therefore also regarded as a reservoir of infection to humans (see, for example, Walker, 1950; La Touche, 1952; Georg, 1954). After the successful treatment of *Microsporum canis* and *Trichophyton mentagrophytes* infections in guinea pigs with griseofulvin by Gentles (1958), Lauder and O'Sullivan (1958) tested this antibiotic on calves exposed to artificial infection with *Trichophyton verrucosum* and obtained similar satisfactory results. Since then veterinary experience with griseofulvin has been gained in the treatment of ringworm in cattle, dogs, cats, chinchillas and chickens. In the results quoted below mycological examination had always been carried out unless otherwise stated.

LARGE ANIMALS

Cattle—*T. verrucosum*

(a) Prevention—Published evidence (Lauder & O'Sullivan, 1958) indicates that 60 mg./kg. given once daily for 5 weeks prevented the establishment of experimental infection in calves, all control animals becoming infected.

(b) Treatment—For treatment, whether experimentally or under field conditions, griseofulvin has produced encouraging results at doses ranging from 30 to 60 mg./kg. for 10 to 20 days. In subsequent trials excellent results were obtained with 40 mg./kg. for 10 days. One week after the end of treatment there was considerable reduction of scabs, and growth of hair. In one trial all animals but one were clear of lesions within 18 days after treatment had begun, as shown in Figures 1, 2 and 3; the rest cleared one week later, except for one animal, which required 15 days' treatment. The scabs, when present, on microscopical examinations showed the presence of fungus which was viable as long as the clinical lesion lasted. Recovered animals appear to have a high degree of resistance to re-infection.

Other trials have indicated that clinical recovery can be observed as early as 7 to 10 days after the beginning of treatment, particularly if the infection is treated early. The natural course of ringworm lesions is said to last approximately three to five months (Ford, 1956). In one trial this period was cut to three to five weeks by the administration of griseofulvin at about 45 mg./kg. once daily for seven days, or even with only four doses at two days' interval; these were only clinical results unconfirmed mycologically.

The results suggest that *T. verrucosum* infection in cattle can be treated efficiently with griseofulvin. So far, however, its use for these animals has not been possible for economic reasons.

SMALL ANIMALS

The incidence of confirmed clinical ringworm infection in domestic pets is low. In dogs the disease can occur as a sporadic outbreak, particularly in kennels. Infection in cats may sometimes be traced through infected human contacts.

1. Dogs—*T. mentagrophytes, T. verrucosum, M. canis, M. gypseum.*

In dogs artificially infected with *M. canis* or *T. mentagrophytes*, daily doses of 25 mg./kg. griseofulvin eradicated the infection in 12 to 14 days (Robinson, 1960). In

FIGURE 1

Cow — before treatment.

FIGURE 2

Cow — after treatment.

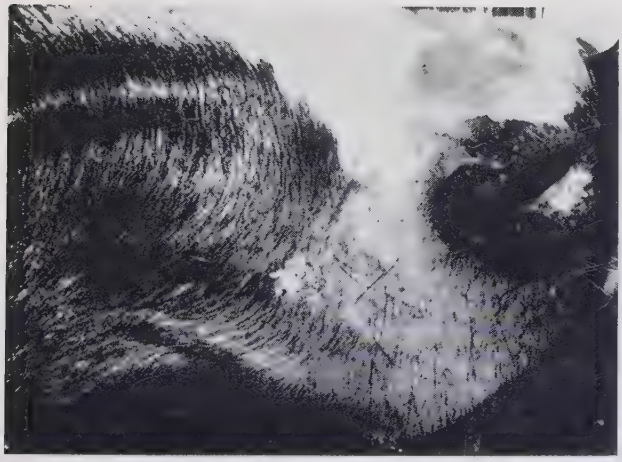

FIGURE 3

Cow — after treatment.

FIGURE 4

Boxer puppy — before treatment.

FIGURE 5

Boxer puppy — after treatment.

FIGURE 6

Chinchilla — before treatment

FIGURE 7

Chinchilla — after treatment.

general, infections by the organisms named above responded well to griseofulvin at a daily dose of 25 to 60 mg./kg. for 3 to 5 weeks as, for example, reported by Lauder (1960). Ablett & Gill (1960) treated four boxer puppies infected with *M. canis* with approximately 25 mg./kg. and obtained a clinical and mycological cure after 28 days of treatment (see Figures 4 and 5). A less satisfactory response in a few animals infected with *M. canis* has been encountered and may even be associated with use of high doses, for a relationship between high doses and poor response has been previously noted in cattle.

2. CATS—*M. canis, T. mentagrophytes.*

Keep (1959) indicated that *M. canis* infection can persist almost indefinitely in some catteries, and that the efficacy of any accepted method of treatment was in doubt. In view of the low incidence of cat ringworm infection in this country, some of the original work with griseofulvin was at first carried out in other countries. For example, Keep (1959) gave two kittens infected with *M. canis* 40 to 60 mg./kg. once daily. The hair was completely clipped and grew quickly, but fluorescence was detected in the hairs until the twentieth day after treatment. After this the fluorescence began to decrease but remained at the tips of some hairs. By the 44th day only a few facial hairs were fluorescing, and these were negative on culture. Kaplan & Ajello (1959) obtained good results on twenty-two cats and kittens at daily doses of approximately 40 to 60 mg. with griseofulvin, depending on the size and age of the animals. Eight cats became mycologically negative, as confirmed by culture, by use of griseofulvin alone; others had antiseptic baths as a surface disinfection. Duration of treatment varied from 3 to 5 weeks.

In this country, infections due to *M. canis* and *T. mentagrophytes* have been treated with griseofulvin in some catteries as have also individual cases by several practitioners, including Bunting (1959), Wright (1959), Lauder (1960), McCormick (1960), and McPherson (1960). In most of these trials the approximate daily dose was 40 to 60 mg./kg. for three to eight weeks. It has been found that response to treatment in Persian cats is slower. Thus, in one cattery all six Persian cats were infected with *M. canis*, but only two showed clinical lesions. On treatment with 40 mg./kg. daily four animals cleared up after six weeks and one after eight weeks; the remaining kitten (the first one to show lesions) was still positive on culture fourteen weeks after treatment and had developed a new lesion.

In another cattery of Persian cats, thirteen out of forty-one cats were infected with *M. canis*, although clinical lesions were only present in four animals and fluorescence in thirteen. All animals were positive on culture. Since animals with clinical lesions were in the same pens as cats that appeared clinically normal, the latter were removed and are being examined at intervals, pending the establishment of suitable conditions for full scale hygienic measures, such as periodic bathing of animals and complete disinfection of pens and cages. Some trials are still in progress.

Sometimes eleven to seventeen weeks of treatment have been found to be necessary; supplementary bathing to eliminate surface infection is also carried out. Probably, because the hair in Persian cats is considerably longer than that of smooth-coated breeds, clipping at the beginning of treatment and frequent brushing may hasten recovery. The lesions in two control animals were completely stationary after seventeen weeks and fully fluorescent up to that time.

There has occasionally been less satisfactory response, and interpretation of results has been complicated by concurrent topical use of other medicaments.

3. CHINCHILLAS—*T. mentagrophytes.*

The disease caused by this fungus is sometimes referred to as "fur slipping" and has been described by Blank et al. (1953). Isolation of the fungus from this infection was subsequently confirmed by Rowsell & Kennedy (1954), Hayes (1956), Kelly & Anthony (1959) and McPherson (1960). The disease has appeared in several herds in Great

Britain during 1959, but its incidence over the entire country is not yet known. Transmission of the dermatophyte from chinchilla to man has also been recorded (Rowsell et al., 1954). Griseofulvin has been found to hasten recovery from this infection (McPherson, 1960), but the view was expressed that for public health reasons the infected animals should either be kept under hospital conditions or be treated with an efficient topical fungicide.

Reports on the treatment of more than sixty animals with 30 to 40 mg./kg. for four to five weeks indicate that clinical results are good (see Figures 6 and 7). Improvement is generally noted during the second week of treatment. Occasionally recurrence of lesions has taken place, particularly if the infection has been treated early in the outbreak of the disease. The questions of development of immunity to the disease after treatment and of the importance of environmental factors require further elucidation.

4. CHICKENS—*Trichophyton gallinae.*

Fowl favus due to *T. gallinae* was apparently common early in the century (Ainsworth, 1954), but it is extremely rare at present (Carnaghan et. al., 1956). Only one outbreak has been encountered, and preliminary results of griseofulvin treatment were not encouraging. Infected hens were given the antibiotic in their food at 40 mg./kg. once daily for four weeks; all failed to respond.

Birds experimentally infected with *T. gallinae,* were treated with 40 mg./kg. and 400 mg/kg. for four weeks there was only a very slight fungistatic effect in some of the birds receiving the higher dose. All treated and control birds in both trials were mycologically positive throughout. The lesions on the former spread rapidly over the remainder of the comb when treatment was stopped.

These poor results may be partly related to inadequate absorption of griseofulvin by the hen. In this connection it is noteworthy that approximately 90% of the administered dose is recovered in the faeces during the twenty-four hours after single oral administration. Only on the higher dose level was the antibiotic detected in the serum after four hours. Further, the particular strain of *T. gallinae* used was not very sensitive to this antibiotic. These findings are based on limited observations, and further work on this species is necessary.

CONCLUSION

Preliminary results suggest that griseofulvin can be of value in the treatment of ringworm infection in animals at an average daily dose of 40 mg./kg., but the duration of treatment depends on the species.

No side effects were shown throughout trials. During administration of griseofulvin to some cats, appetite was greatly increased, and in particular kittens grew very quickly. This effect has not been checked experimentally. Further chronic toxicity tests indicated that griseofulvin fed for one year to dogs at about ten times the therapeutic dose had no observable detrimental effects on the animals.

ACKNOWLEDGEMENTS

I thank many of my colleagues in practice and within our own organisation for collaborating in some of the work reported. Special thanks are due to Messrs. Lauder and O'Sullivan for their original work on griseofulvin, to all clinicians who have sent communications of their results and in particular to Mr. P. K. C. Austwick for his services to veterinary mycology. Photographs were taken by Mr. D. Boxall.

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DISCUSSION

Dr. Sharvill: At the request of various veterinary surgeons I have treated sixteen cats and kittens infected with *M. canis*. These all had naturally acquired symptomless infections demonstrable by Wood's light. Some had previously had visible lesions and had been declared cured by veterinary surgeons, but had subsequently infected children. Positive cultures were obtained in all cases. Griseofulvin was given in a daily dose of 250mg., for periods of two to twelve weeks. Results were on the whole unimpressive, although most of the animals ultimately recovered, and this raises a difficult question. Even the most eminent veterinarians are unable to give me factual information about the normal course of ringworm in kittens. Do they, for example, get better at puberty? In one household, I treated four Siamese kittens, litter mates, who made little response, but later on their fluorescent hairs disappeared just when they started to call, that is, at puberty. Living with them was a ginger 'alley cat' who had fluorescent whiskers which resisted three months of griseofulvin. This cat suffered from a gynaecological disorder for which she underwent oophorectomy; a week or two later her whiskers had ceased to fluoresce. I did not wash these cats, nor did I dust them with Captan, as suggested by Dr. La Touche. I have used this fungicide on roses, but not on animals.

Dr. La Touche: That is my experience over a number of years. Many cats recover from this within a period of from two or three months to six months or several years. I have had one for three years and eventually cleared him up with tincture of iodine; there is just one lesion left. This cat remained infected despite all attempts at topical treatment. In those days, of course, we did not have griseofulvin, or we did not know it could be used orally.

As far as the normal course of ringworm in cats is concerned, I find that it varies very much according to the time at which you get the cat. You obviously do not know when the lesions started, and you have to take all these things into consideration when you undertake treatment. A lesion which clears up quickly may be one that is just about finished anyway, whether you treat it or not.

Miss Uvarov: In defence of the cat, in this particular series of Persian cats which I am treating it is a bit too soon to give you all the information, because my controls are still positive at three months; they are still infected clinically and the lesions have not altered in any way. I think Heap in Australia has indicated, and agrees with Dr. La Touche, that infection in the cat can persist for at least three to six months in its clinical form. So that we do feel that by having these trials with griseofulvin in that species we may be able to save the lives of some of these cats, because before the advent of griseofulvin many of them were destroyed.

Prof. Riehl: It may be interesting to Dr. La Touche to hear that I have two cats, and one of them has severe lesions all over its body. I did not treat the cat because I wanted to see whether there was a possibility of spontaneous healing; after a few months everything disappeared without any treatment.

Dr. Barlow: Miss Uvarov referred to certain cats which relapsed when treatment was stopped. Might I ask her if she noted by which method reinfection took place? Was it new surface infection, and was there any sign of extension down previously infected hairs which had been clearing? If the latter occurred it would throw some light on the matter we were talking about earlier, as to whether there is a band of resistant keratin formed in the hair.

Miss Uvarov: The cases of relapse are actually very few; there have only been two so far. Again, the groups are rather small, but in one group of six cats four were clinically and culturally negative after six weeks, one after eight, and one was still infected at eight weeks and at ten weeks developed a new lesion away from the original lesion. All the others are still under treatment. I am afraid the only other relapse was in one cattery where bathing was being carried out and various medicaments were being used as well.

Dr. Williams: There is some evidence in human scalp infection that griseofulvin will not touch hairs which are in a resting phase. I know very little about the moulting of cats, and still less about when the whiskers come off, but I wonder what part, if any, this feature of the disease might play in the fact that these cats do not get better. Are their hairs in a resting phase and not being touched by griseofulvin?

Dr. La Touche: I do not know whether this will answer your question, but I do find that in lesions of cats which I have treated with griseofulvin some hairs show fluorescence at the tips and others show fluorescence all along the extent of the shaft. This suggests that there is some difference in behaviour between individual hairs in any particular lesion and the effect of griseofulvin is not uniform. I think that has been the trouble with my cats, that all the hairs were not affected uniformly by the griseofulvin.

Dr. Moynihan: It would seem rational, in answer to Dr. Williams' point, that you could always bring a hair follicle into activity by plucking and, therefore, the practice in the past of depilation was doubly effective because it removed the infected hairs and also brought the resting hairs into active growth so that a substance such as griseofulvin could be incorporated in the growing hair.

I would also like to ask Dr. La Touche how common is infection of the vibrissae of the cat. I remember one family that was infected where the source of infection was just one vibrissa. We removed it and that seemed to do the trick. I do not know if they are particularly liable to get infected.

Dr. La Touche: I cannot answer that question accurately, but I would say that in about three or four per cent of cats the whiskers become involved. It is not very frequently.

The Chairman: Dr Frey, I believe you have a few slides to show us.

Dr. Frey: It may be interesting, in relation to the actual discussion, to show our findings working with experimental infections of guinea-pigs. We were astonished by the fact that, in prophylactically treated animals, ten days before the inoculation and twenty days after it—that means thirty days after the beginning of treatment—we found culturally positive hair, that is, positive cultures in the scales and hairs of these animals. For this reason we treated the animals locally, and this was the only way to obtain immediately negative cultural findings during the whole evolution of experimental infection.

This brings up the problem of the permanence of griseofulvin in the hair. If you treat the animals intermittently each second or each third day, you have to raise the dose very much to obtain results. You have to give 200 mg. per kg. to obtain the same results with intermittent treatment as you would get with 50 mg. if you gave it every day. It is important to mention in this connection the experiments of Rosenthal, who could get positive infections of guinea-pigs which had been fed griseofulvin for two weeks before; the feeding had then been interrupted, and after three days they started showing inflammation. So the problem of how griseofulvin is incorporated in the hair must be considered. Possibly it is kept in as long as the blood level is high, but is lost the moment the blood level falls.

Dr. Calnan: I think Dr. Williams' point is a very relevant one. Epilation treatment shows that hairs in the resting phase cannot be infected with the ringworm fungus. If a hair that happens to be infected with the fungus goes into the resting phase, presumably it will be spontaneously cured. One wonders, in the particular case of Miss Uvarov's cat, whether that individual whisker had gone into the resting phase. It would have been interesting to pluck it out and see whether it was a clubbed hair.

The other thing I would like to say is that the remarks which have been made about spontaneous healing and the ease of curing *Microsporum canis* infection in kittens and cats apply equally to dogs. I think in the past we have been too ready to advise that infected dogs should be destroyed. I have known a number of cases where, for sentimental reasons, attempts have been made to treat them locally and have met with quite a high rate of success.

MISS UVAROV: I would like to take up this point, and particularly the last statement that perhaps in the past animals have been destroyed unnecessarily, or perhaps because of the danger of human contact. It is extremely easy to perform euthanasia on small animals, but it is rather hard to bear, and I know some children and old people have been very hurt when the doctor has said that a ringworm infection must be from the cat or the dog. I think in the past there have been difficulties in obtaining confirmed diagnoses. I hope that from now on any doctor who suspects a cat or a dog will give the same consideration to my patients as he does to his own. What is more, I would be even more grateful if some of the mycologists present would give the same facilities for cultural diagnosis to pets as they do to mere humans.

As to this particular whisker which proved to be so stubborn, I had to be rather selfish and keep it there for a bit because I wanted to know if it was going to break off or be shed. Some of these infected whiskers do break off and eliminate themselves this way. In the end I did have to pull it out. Unfortunately, speaking from memory, there were two or three other whiskers that were pulled out and examined, and I have an idea this particular one was negative on culture, but I am not absolutely sure. In some cases it does mean pulling these stubborn whiskers out.

DR. LA TOUCHE: May I ask Miss Uvarov whether she kept controls in the case of dogs, or whether she just treated them as they came, as I used to do?

MISS UVAROV: Yes, I have kept some controls in a pack of hounds affected with Trichophyton mentagrophytes. Unfortunately I think I came in too late, because all the treated animals responded extremely well. There were about eight treated and six controls, and two of the controls did as well as the treated except that one of them was still positive culturally after twenty-eight days. That is about the only outbreak where I have been able to keep controls, because if the animals are not under hospital conditions people are very frightened of keeping controls. In this particular instance it did not seem to matter very much; although the hounds were galloping all over the countryside, but the owner was co-operative. My impression was that the treated animals did do a little better than the controls, considerably better, really, because the lesions regressed quickly. But I think I was a bit too late in the outbreak. I have had no controls at all with *Microsporum canis* infection.

DR. LA TOUCHE: My experience was that all you had to do was to give the dog a bit of ointment and in three weeks' time it was all right; they responded very well. I think the reason is that these lesions with *Microsporum canis* are much more inflammatory on the whole than the type of lesion we get in a carrier cat. There has never been any question of destroying the dog, except for one occasion when I wanted histological material.

THE CYTOLOGICAL EFFECTS OF GRISEOFULVIN

P. C. Koller

Paget and Walpole (1958) reported that griseofulvin produces a colchicine-like effect on dividing cells. They found that, besides temporarily arresting mitosis in the metaphase stage, griseofulvin affects the movement of chromosome at anaphase, which may lead to cell degeneration. The aim of the present paper is to describe the cytological effects of griseofulvin, on the crypts of Lieberkühn, germinal epithelium of seminiferous tubules, haematopoietic marrow, and experimental animal tumours. The results of tests for carcinogenicity on griseofulvin carried out during the last two years will be reported also.

Our results confirmed Paget and Walpole's finding, i.e. a dose of 200 mg./kg. griseofulvin is required to produce cytological disturbance. The antibiotic was ball-milled for 24 hours and suspended in Tween 80 wetting agent before being injected intravenously into rats (120-140 g. body weight). Ball-milling of the griseofulvin powder is essential: it reduces particle size to about 4-6μ, reduces intravenous toxicity, and facilitates the transport of griseofulvin and enhances its absorption by cells.

After the intravenous injection mitotic disturbance was observed in the intestinal epithelium: it was manifest also in the marrow cells in about six hours, and in the testes about seven hours after injection. It was found that cell division was arrested at the metaphase stage, the chromosomes being scattered: in some cells they formed 2-3 distinct groups. The position of the chromosomes in these cells showed that the drug's action differs from that of cholchicine. The latter suppresses spindle formation but does not interfere with the normal metaphase orientation of chromosomes.

It is interesting to note that the chromosomes of the affected cells were undamaged, and no fragments were observed when the cells had recovered from the effect of griseofulvin. The large number of degenerating cells, which were seen 24-28 hours after griseofulvin treatment, may be attributed to the abnormal distribution of chromosomes, caused by the irregular mitotic spindle.

The effects described above were similar in every rat tissue in which mitosis was in progress. However, the consequences of mitotic disturbance were different in various tissues, and will now be described.

In the *intestinal mucosa,* where mitotic rate is highest, recovery from the effect of griseofulvin was quickest. Within 24 hours mitotic rate in the crypts of Lieberkühn was normal, and very little evidence of cell degeneration could be seen. If it were possible with griseofulvin to produce a drastic arrest of mitosis lasting 24-48 hours then degeneration of a large number of dividing cells would ensue and lesions would develop in the gastro-intestinal tract, with very serious consequences. It is known that a damaged intestinal epithelium can result in bacteremia and death within 3-4 days. No such death occurred in our experiments, which indicates that the lesions—if they occurred at all—were repaired.

In the *femoral marrow* of rats, griseofulvin had similar affects on all cell types. Special effort was made to determine whether the recovery rates for cells of the lymphoid, erythroid and myeloid series were the same or not. It appeared that cells of the myeloid series were the slowest in recovering from the effect of griseofulvin. The rats' femoral marrows were normal 72-96 hours after treatment.

The most interesting cytological after-effects of griseofulvin were observed in the *testes.* Unilateral gonadectomy made it possible to study the consequences of mitotic and meiotic arrest. It was observed that cells of the germinal epithelium in the seminiferous tubules recovered and became fully active within 48 hours of injection. In tubules, however, where spermatogonial cells were arrested in mitosis, maturation and meiosis of primary spermatocytes were delayed. This resulted in depletion of the germinal epithelium in these particular seminiferous tubules (Fig. 1). The arrest of meiosis

in the primary spermatocytes and the abnormal chromosome segregation resulted in the spermatids failing to differentiate, consequently the lumen of some seminiferous tubules and epididymis were filled 5-6 days later with degenerating or incompletely differentiated germ cells (Fig. 2). Fourteen days after griseofulvin treatment the histological appearances of testes and epididymis were normal, as shown in Figs. 3-4.

The effect of griseofulvin on *transplanted tumours* was studied also. Rats grafted with Walker carcinoma were given 200 mg./kg. griseofulvin intravenously and sacrificed 5, 7, 24, 48 or 72 hours later. The maximum effect was observed at 7 hours, when the frequency of dividing cells in the tumour was about 18 per cent, i.e. six times higher than in a control tumour. At forty-eight hours the mitotic rate had reverted to the normal level of 2·5-3 per cent. Growth of the Walker carcinoma was not inhibited by a single dose of griseofulvin and cytological analysis of the tumour showed that mitotic arrest and subsequent degeneration had occurred in available cells. For a 3-4 day temporary inhibition of growth to occur in this particular experimental tumour, at least 40 per cent of the dividing cells must be eliminated: 75 per cent destruction is required for 6-8 day cessation of growth. The 200 mg./kg. dose of griseofulvin was insufficient to produce even a temporary halt of 3-4 days duration in tumour growth.

Attempts were made to inhibit tumour growth by administering the drug daily. In one set of experiments intravenous doses of 50 mg./kg. were injected once daily on four consecutive days, and in another experiment 4 doses of 150 mg./kg. were given for two days. In neither of these experiments was tumour growth inhibited and it is concluded that griseofulvin is not effective against this particular experimental tumour.

TABLE I

Test for Carcinogenicity on Griseofulvin using Mice and Rats

SPECIES	STRAIN	Treatment	Number of Animals	Number of Tumours	Duration of Experiment (in months)
MICE	SWISS	BP. (1)	20	2	8
		V (2)	60	Nil	12
		GF. (3)	60	Nil	12
	CBA	BP. (1)	10	2	15
		GF. (3)	20	Nil	15
	S-CB	BP. (1)	5	1	15
		GF. (3)	5	Nil	15
RATS	WAG	V. (2)	60	Nil	12
		GF. (3)	60	Nil	12
		Control	60	Nil	12
		GF-F (4)	60	Nil	12

(1) BP: 3-4 Benzpyrene, 1 x 20γ in 0.1 ml. oil, s.c. inj.

(2) V: vehicle: Sodium-stearate, 25 x 0.04% in 0.1 ml. aq. s.c. inj.

(3) GF: Griseofulvin, 5% in vehicle s.c. inj.

(4) GF-F: Griseofulvin, 1% in diet for 12 months.

Studies were also carried out to detect possible pathological or *neoplastic effects* which might develop either locally or systemically as a result of chronic administration of griseofulvin.

Mice and rats were injected subcutaneously once weekly for six months with griseofulvin (250 mg./kg.). The drug was suspended in 0.04 per cent sodium stearate when given to mice, and in 0.2 per cent when injected into rats. Animals were sacrificed at various time intervals, and the intestinal mucosa, marrow and testes were cytologically and histologically examined. The observation covered 16 months. No pathological lesions were observed in these tissues either during or after treatment of the animals with griseofulvin.

In another set of experiments, rats were kept on diet containing griseofulvin (1%) for twelve months. Histological studies of these rats revealed no gross pathological effects in testes or gastro-intestinal tract. Some rats developed thyroid hyperplasia, which was characterized by changes in the follicular epithelium and lack of colloid.

The carcinogenicity test was carried out on mice and rats. Three strains of mice, Swiss, DBA and S-CBA were used: their age at the onset of treatment with griseofulvin was $2\frac{1}{2}$ months. To test the sensitivity of these strains to carcinogenic stimuli, single doses of 20μg of 3-4 benzpyrene in 0.1 ml. of oil were injected subcutaneously. A few animals developed tumours at the site of injection within three months, indicating that the mice of all three strains were capable of responding to the smallest dose of a known carcinogen. No tumours were produced when either the vehicle (sodium stearate) or griseofulvin was administered once weekly for 25 weeks. The results of the carcinogenicity test on rats were negative also (Table 1).

Conclusions

Griseofulvin, when administered intravenously, produces a temporary arrest of mitosis in the intestinal epithelium, haemopoietic tissues and germinal epithelium of the testes. A dose of 200 mg./kg. is required to induce such effects in rats. The recovery of these cells from mitotic arrest is very rapid, and normal cellular activity is restored within a few days. No carcinogenic activity for griseofulvin was revealed by our experiments, in which mice and rats received 25 weekly injections or were kept for twelve months on a diet containing one per cent griseofulvin.

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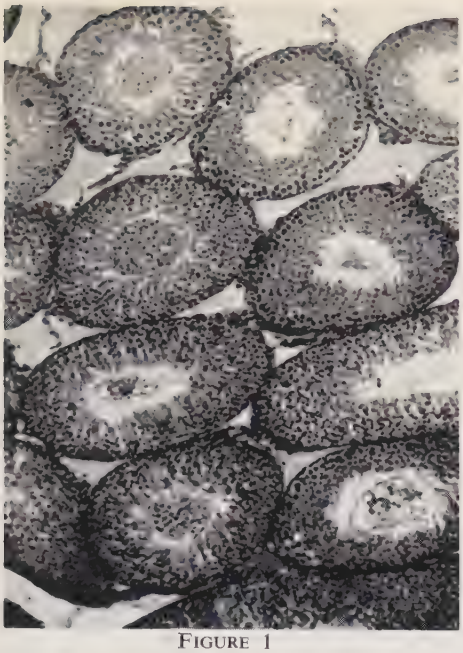

FIGURE 1
Seminiferous tubules in the testis of a rat six days after a single intravenous injection of griseofulvin (200 mg./kg.). The germinal epithelium is depleted and sperm differentiation is absent in some tubules. × 100.

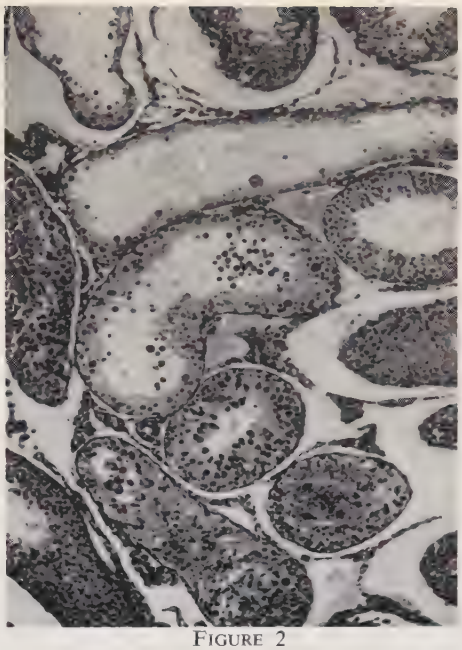

FIGURE 2
Epididymis of a rat six days after treatment with griseofulvin. The lumen are filled with degenerating and undifferentiated spermatids. × 100.

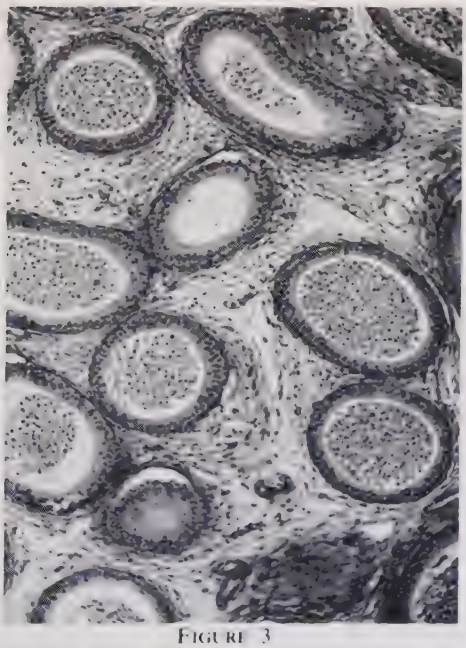

FIGURE 3
Testis of a rat 14 days after griseofulvin treatment, showing mainly normal spermatogenesis in the seminiferous tubules. × 100.

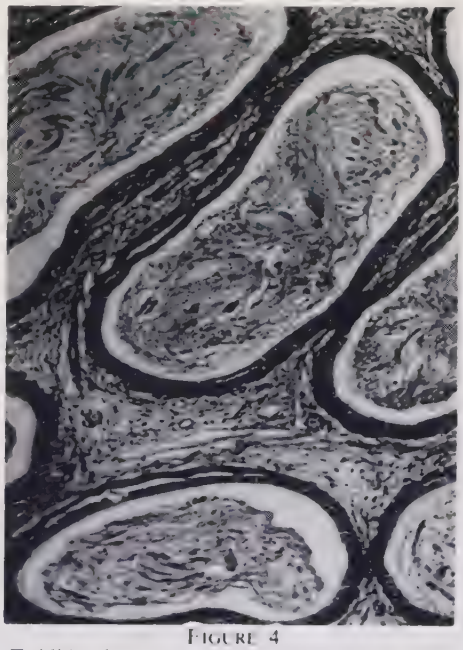

FIGURE 4
Epididymis of same rat, showing well-differentiated spermatozoa. × 100.

DISCUSSION

DR. CHATTAWAY: Could I ask Professor Koller, in his long-term feeding experiments with rats, whether any have been done with young rats, and whether the long-term administration of griseofulvin had any depressive effect on the general growth of the animal, rather than on the reduction of tumours?

PROF. KOLLER: The rats were about two and a half or three months old when the experiment started. These are long-term experiments so we do not want the animals to be too old because then we run into other difficulties. We have to have young ones because they develop so many other pathological lesions. These were quite young animals. I believe that Glaxo Laboratories have been carrying out experiments for the very purpose which you mention. I did not myself see any deleterious effect, but I was not particularly interested in this. Perhaps colleagues who were working along these lines at Glaxo Laboratories could give you a more satisfactory answer. My experience was that there was no deleterious effect on the growth rate. As a matter of fact, I believe some animals did better on the griseofulvin diet than on the normal diet.

DR. MOYNIHAN: Would Professor Koller suggest using griseofulvin for human chromosome cell counts?

PROF. KOLLER: Yes, it would be very good if we could replace the colchicine. We have been using that injected in man, and we have then taken out the marrow for some studies. There is not so much individual variation as we have found after griseofulvin treatment in animals. The griseofulvin treated animals' cell divisions are not so normal. It is not an imitation of the colchicum effect; it is a much more crude effect which is produced on the cell, as Dr. Walpole described. The chromosomes do not lie beautifully in the metaphase plate so that the cells could be processed in this state, but the chromosomes are scattered all over the cell. It was even suspected that there might be multiple forms where the chromosomes are pulled in different directions.

I use griseofulvin to frighten people about cancer, to show what an enormous number of cells there are in division and why, for instance, early diagnosis and treatment at once is so essential. When cancer is diagnosed the patient should, within twenty-four hours, either be on the operating table or under treatment because of that. Griseofulvin can really show that beautifully; it is excellent for showing how many cells there are in division within five hours.

THE CHAIRMAN: Have you any experience of massive doses of griseofulvin on existing tumours?

PROF. KOLLER: We tried it on several tumours, even on spontaneous tumours, and we did not get any inhibition. To get inhibition we should need to continue with the very heavy dose given intravenously. The smaller doses produced no effect whatsoever.

THE CLINICAL VALUE OF GRISEOFULVIN

David I. Williams

We meet to celebrate, among other things, the coming of age of griseofulvin, for it was in 1939 that Raistrick and his colleagues worked out its structure. It was, of course, very much of a test tube baby, albeit of quite cultured parents, but of such a large family that, apart from the inevitable proud but restrained remarks of its parents, its identity tended to get lost. At the age of seven or eight griseofulvin again began to be noticed, particularly by its godparents (Brian et al. 1946, Grove and McGowan 1947) as someone a little unusual. He had to pass a number of stiff examinations and there was some difference of opinion amongst some of the examiners as to his real ability. Despite his slightly checkered career, he was admitted as an interesting and promising candidate to Glasgow University. At the age of nineteen he was modestly hailed by Dr. Gentles (1958) as one able to do things not before reported. One or two more stiff examinations and a few complaints that his brilliance might even have blinded the examiners into passing him too easily, and he was out in the great big world, his teenage period of uncertainty over, and the future apparently bright. After a probationary period of some six months, he has now been earning his own living, as it were, for over a year. At the age of twenty-one we can now better assess his worth and, indeed, begin to see some of his weaknesses.

I do not want to weary you with a lot of charts and figures which, with due respect, I suspect you would not remember. I should prefer, if I may, to discuss the matter in the general rather than in the particular, although I will, from time to time, have to draw on our experiences at King's College Hospital. I ought to say here that we have only treated two hundred and eleven cases of dermatophyte infection of various sorts. Break these down anatomically and according to the fungus involved or the type of disease and the sub-groups are very slight. Since it is now reasonably clear that griseofulvin is a remarkable advance in the treatment and, often, cure of dermatophyte infections my interest now is more in those cases where it seems to fail and in seeking reasons for this failure. Since in this country we have never been manic—only pleasantly euphoric—about the success of griseofulvin, there is no need for a swing into a mood of depression.

Perhaps the first indication for the use of griseofulvin is ringworm of the scalp. I have hardly any business even to discuss this matter, since we have only treated fourteen cases. Some months ago it was not sufficiently appreciated that infection was likely to persist unless the involved hairs were removed. It seems to me sensible to snip off or shave off the infected parts of hairs when they have grown clear of the scalp, which takes about three weeks. Local clipping or shaving is feasible only when the extent of the disease can be seen under Wood's light. In the non-fluorescing cases the whole scalp is probably best shaved at three weeks. Local treatment with an anti-fungal preparation has much to commend it, continued for a week or two after apparent cure to prevent re-infection from odd spores lying about. Griseofulvin having been given does not prevent later re-infection from hats, caps and the like. Grin (1959) of Sarajevo suggests that difficulty may arise from infection persisting, as he has shown it can, in hairs in the resting phase. I am not sure to what extent this is of practical importance.

I will turn now to nails. We think we have cured thirty-two out of forty-nine patients suffering from a Trichophyton rubrum infection of the finger nails and most were well in six months. Two others, however, are no better at six and eleven months respectively. The rest seem to be doing, as Ward Sisters say, as well as can be expected. One patient with Epidermophyton floccosum infection is still manifestly infected after fourteen months of treatment. If people are either catchers or non-catchers of ringworm, as seems to be true for many parts of the skin, how soon shall we discover whether the

catchers are also re-catchers? Already one of our earliest finger nail cures again has evident disease, starting as a little ridging in the distal but central part of one nail five months after stopping griseofulvin and one year after what we called cure. Toe nails are ridiculous things by any standards. To prove the presence of infection is not always easy and to decide when cure has taken place can be even more difficult since, even without infection, because of previous trauma or over-enthusiastic avulsion nails may be permanently thickened and dystrophic. Mycological positives may be infuriatingly elusive. This is an important point since failure properly to recognise that infection may be persisting, even in a tiny apology for a little toe nail, may entirely vitiate findings when one day we come to discuss the difficult question of re-infection or relapse. Toe nails grow so slowly, except in the young, that rapid cure is not to be expected. Indeed, this factor of slow growth may be one of the things preventing cure. We have treated, or are treating, nearly one hundred patients for toe nail infections. Sixty-seven of these have proven Trichophyton rubrum infections and we think that we have cured only nine. Twenty-three others seem to be improving and seven of these have already passed a year of treatment. It must be remembered that many months, sometimes even a year, may pass before there is obvious clinical evidence of improvement in infected toe nails. Thirty-five of the sixty-seven cases are as yet unchanged, but fifteen of them have not yet received six months' treatment. It is curious how sometimes all the toe nails bar one may seem to be cured, or all the nails on one foot but none on the other or, without rhyme or reason, some toe nails get well and others don't. Sometimes as weeks and months go by infection can be seen now to be regressing and now advancing. The fungus can be found—or, to be accurate, Dr. Gentles can find it for us—until the last bit of diseased nail is grown out with no greater difficulty than at the beginning. Toe nails, then, don't do very well. Avulsion of nails combined with griseofulvin by mouth leads to more certain and quicker cure. New nails grow with surprising rapidity after removal of the old.

Ringworm of the body regularly seems to do very well, as does *tinea cruris*, whatever the organism. In favus, Tokelau ringworm and some of the Trichophyton rubrum infections griseofulvin has proved a miracle drug. For the rest, it is an elegant treatment; after all, inflammatory cases have not presented a big problem even in the pre-griseofulvin era.

Blistery eruptions on the palms and soles, whatever the organism, also respond well but not rapidly. Cure cannot be accepted until the last remnant of ruptured blister has been shed and this takes about a month. Scaly hyperkeratotic palms, again whatever the organism, do well, although sometimes rather slower than the expected six to eight weeks. On the other hand, soles of this type are more difficult. For instance, of fifty-four cases of Trichophyton rubrum infection treated for at least three months, forty-nine seem to be cured and remain so; but it must be remembered that most of these are still taking the drug for nail infections. One case was not cured for ten months. The rest are, as it were, still with us. One still persistently has Trichophyton rubrum present after forty-four weeks' treatment, another after twenty-nine, another after seventeen and another after fourteen weeks. Five patients, having become in every way clear—and our criteria are very strict—grossly relapsed while still taking griseofulvin, two at four months and three at six months. Three of these again have appeared to become clear, but two obstinately refuse to get well. Three patients have erratically persistent infection, combined with erratic treatment. Finally, one of what I have been pleased to call cures has either relapsed or been re-infected five months after the end of an eleven month course of treatment; his toe nails are not yet obviously re-infected.

I do not account for the relapses while the patients are on regular treatment. Three were men with nylon socks, one with those monstrosities under army boots and all five had very sweaty feet. I will return to this question in a moment.

Finally, to the devilish mystery of the cloven hoof; by which tortuosity I mean the interdigital clefts. It will be only too well known to you that every lesion between the toes is not a fungus infection, even though often treated as such: equally, you will

know that even with no treatment fungus infections between the toes may be here today and, seemingly, gone tomorrow. To say that infection in this site is cured requires a certain boldness, if not foolhardiness, however easy it may seem to some to laugh off reappearance of disease as a fresh infection. But if patients are taking griseofulvin constantly for long periods, usually for nail infections, one would expect interdigital infection to disappear and re-infection not to happen. But that is not the case, certainly not in our series, although it must be remembered that these were cases usually with widespread and long enduring disease not limited to the clefts. I believe it makes no difference what organism is concerned, but let me quote the Trichophyton rubrum figures from our series. Fifty-nine cases were found at some time or other during treatment to have mycological cleft infection. I think we have cured only ten of these. Certainly thirteen had laboratory evidence of infection after over three months' treatment, seventeen after over six months and six after over a year. Obviously if this problem cannot be overcome we are not going to cure any patients completely to our own satisfaction. Of course, in a cynical moment you may say that they are all going to get re-infected anyway. Why should infection be so difficult to eradicate in this area? Perhaps for the similar, but unknown, reasons that relapse or persistent infection also occurs on the soles. I can only ask questions and hope that somebody will tell me the answers. Do other organisms flourish in these cases which break down griseofulvin? Is there some other factor produced in these areas which kills griseofulvin? Does sweat itself perhaps wash out griseofulvin from the keratin, although this seems a singularly naive suggestion. It is of a little interest that griseofulvin can be recovered from cotton wool plugs placed between the toes of patients for twenty-four hours in total amounts which varied in six patients we treated from four to twenty microgrammes. One can, of course, recover relatively large amounts of griseofulvin in sweat from any part of the body. When I use the term griseofulvin I am quite aware that we do not know what the substance is in keratin which is hostile to fungi, although we may have given griseofulvin by mouth.

We most carefully avoided for a long time any ancillary treatment in our cases so as to give griseofulvin alone its proper chance. More recently, inevitably, a local application has been added to some of these recalcitrant clefts. Most of you will know that topical griseofulvin was tried by several people four or five years ago and found wanting. There is as yet no evidence which has convinced me that local treatment is effective in any other way but by causing an inflammatory reaction. There is, perhaps, a place for a prolonged and full trial of griseofulvin in some of the newer vehicles in some such institution as a boarding school. Of fifteen patients with sole and cleft infections only two thought that griseofulvin lotion as supplied by us was of any help at all, but these are yet early days.

We know that griseofulvin in a dose of one gramme a day, whether in divided doses or not, is reasonably satisfactory. Theoretically even a single dose ought to produce a cure—and has done so in practice in many cases of ringworm of the scalp—but in other sites failure is virtually the rule, perhaps because of re-infection behind the wave of griseofulvin. In my experience patients whose treatment has been short or irregular do not get well, although, for instance, one or two nails will get well and others will remain infected, or nails get well and the soles no better. Reduction of the dose below one gramme on a single day, except in the very young, has mostly proved disappointing. On the other hand, doses at longer intervals seem promising; two grammes all at once twice a week is our present regime and it seems to me as good as one gramme daily in the Trichophyton rubrum cases. Ought we to increase the dose for the cases which fail to respond? There is room for much experimentation and, indeed, trials of different dosage schemes are going on all over the world. The results are sometimes difficult to interpret because ancillary treatments have been applied, and some of the trials have been short in time and material.

Finally, what about the side effects of the drug? Early anxieties have been allayed. A better understanding of the wide variations in the normal blood count, the reporting

of normal sperm counts and testicular biopsies, and the realisation both by patients and doctors that griseofulvin gives no immunity to the normal ills our flesh is heir to, allows us to prescribe the drug with as little anxiety, perhaps even less, as when we order aspirin. I have not ordered a blood count since June of 1959. Headaches do occasionally happen: griseofulvin enhances, if that is the word, the effects of alcohol—but, lest you be tempted, not very often. I believe that it may cause toxic eruptions—in our one case, urticarial, regularly reproducible by griseofulvin but not by dummy tablets. We have stopped treatment in five cases because of severe and continuing diarrhoea; but how do you decide whether a drug is the cause of diarrhoea? However, I feel sure that at least three of these were due to the drug. The other side effects are trivial in the main and rarely long continuing.

What of the future? There is much to be done if we are intelligently to follow the career of this youngster, particularly if we are to solve the mysteries of the failures. Some time we may celebrate another anniversary in the world of mycology. Will it be of griseofulvin, or is there a more brilliant and more powerful half-brother, as obstetricians say, on the way? To change the metaphor, there are no obvious classic fillies in the field as yet.

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REPORT ON A CLINICAL TRIAL WITH GRISEOFULVIN

W. Frain-Bell and C. J. Stevenson

In January 1959, a clinical trial of the efficacy of griseofulvin in the treatment of ringworm infection of the skin and nails was started at St. John's Hospital for Diseases of the Skin, London. It began with a preliminary study of 12 in-patients whose progress was subsequently followed in the out-patient department for a year. The second part of the investigation was a double blind trial in which a further 76 patients took part. The object of the trial was to assess the efficacy of griseofulvin and to consider the significance of any side effects encountered. Details of the trial have been published by the authors in collaboration with Dr. Brian Russell, Dr. R. W. Riddell, Miss N. Dzawachiszwili, and Dr. S. L. Morrison (Russell et al. 1960).

PRELIMINARY STUDY

Firstly, twelve patients were admitted to hospital for one month; all had extensive infection with *Trichophyton rubrum* except two in whom the fungus was *Trichophyton mentagrophytes var. interdigitale*. After one week during which they received inert tablets, treatment was started with griseofulvin 0.5 gramme thrice daily, continued for three weeks in the wards and subsequently under out-patient supervision. One of the the twelve patients developed scarlet fever and was then removed from the trial.

Case No.	Patient	Before treatment	After 17 days' treatment	After 1 year's treatment
I	J.D.	+ (18 mm.)	+ (14 mm.)	+ (6 mm.)
II	M.D.	+ (12 mm.)	+ (10 mm.)	+ (10 mm.)
III	P.G.	+ (15 mm.)	+ (8 mm.)	+ (14 mm.)
IV	E.L.	+ (15 mm.)	+ (9 mm.)	− (0 mm.)
V	H.W.	+ (10 mm.)	+ (8 mm.)	+ (6 mm.)
VI	F.H.	+ (10 mm.)	+ (8 mm.)	+ (6 mm.)
VII	L.M.	+ (12 mm.)	+ (8 mm.)	+ (10 mm.)
VIII	K.W.	− (0 mm.)	− (0 mm.)	− (0 mm.)
IX	B.J.	− (3 mm.)	− (3 mm.)	− (0 mm.)

TABLE I

Trichophytin reactions recorded before and during griseofulvin therapy in 9 patients. All patients had *T. rubrum* infection except Case 3 (P.G.) who had *T. mentagrophytes var. interdigitale*. Immediate reactions only are recorded as diameter of wheals in mm.

Trichophyton tests were carried out by one of us (C.J.S.) on 9 patients before treatment, after 17 days of treatment, and one year later (Table I). Positive immediate reactions occurred in 7 out of the 9 patients tested. At the end of one year, only 1 of the 7 patients gave a negative reaction, this occurring in the only case in which all mycological tests were then negative. There was a reduction in the diameter of induration produced at the end of one year in the remaining 6 patients. Only one patient produced a delayed positive reaction which was again positive at the end of the year.

Case No.	Patient	Finger nails		Toe nails	
		Appearance	Mycology	Appearance	Mycology (direct) examination
1	E.L.			Normal (from 9 mths.)	Negative
2	F.S.			Normal (from 7 mths.)	Positive (sole and toe webs also positive)
3	M.D.	Dystrophic	Negative (from 7 mths.)	Dystrophic	Positive (toe webs also positive)
4	L.M.	Normal (from 7 mths.)	Negative	Dystrophic	Positive
5	P.G.	Slightly dystrophic	Negative (from 7 mths.)	Slightly dystrophic	Negative
6	A.D.	Normal (from 7 mths.)	Negative	Dystrophic	Positive (toe webs also positive)
7	F.H.	Normal (from 5 mths.)	Negative	Slightly dystrophic	Positive (toe webs also positive)
8	H.W.	Normal (from 8 mths.)	Negative	Dystrophic	Positive
9	B.J.	Normal (from 8 mths.)	Negative	Dystrophic	Positive
10	W.I.+			Dystrophic	Negative
11	W.2	Normal (from 7 mths.)	Negative	Dystrophic	Positive

TABLE II

Preliminary study. Results after 1 year in 11 patients receiving griseofulvin.
* Therapy stopped after 7 months.

After 1 year, of the 11 patients studied 8 still showed fungus in toe-nails, 3 in toe-webs, and 1 on the soles (Table II). None, however, showed fungus on the palms, and the finger-nails had become mycologically negative in 8 affected patients within 8 months.

DOUBLE BLIND TRIAL

Description of Trial

Following the preliminary study of the effects of griseofulvin in 12 patients, it was decided to carry out a separate clinical trial on out-patients. The object was to study the therapeutic and side effects of the drug over a prolonged period by comparing a group of patients treated with griseofulvin in a dose of 0.5 gm. thrice daily, with a control group given placebo tablets* of similar appearance and taste. To avoid bias, a double blind form of trial was used in which neither physicians nor patients knew whether treatment was with griseofulvin or placebo tablets. No local treatment was given. A serial number was allotted to each patient when first seen, and on arrival at the dispensary the pharmacist decided whether the patient should have griseofulvin or placebo tablets. He consulted a pre-arranged list allocating patients at random according to their serial numbers to the treated or control groups. New patients were admitted to the trial at weekly intervals, clinical observations were made monthly and submitted to analysis by Dr. S. L. Morrison, the statistician in our team. The first analysis was carried out to decide when to stop the intake of new patients. This sequential analysis was based on the first finding by either of two assessing physicians of "definite clinical

* Containing calcium phosphate (215.5 mg.), starch (55.5 mg.) and magnesium stearate (4.5 mg.)

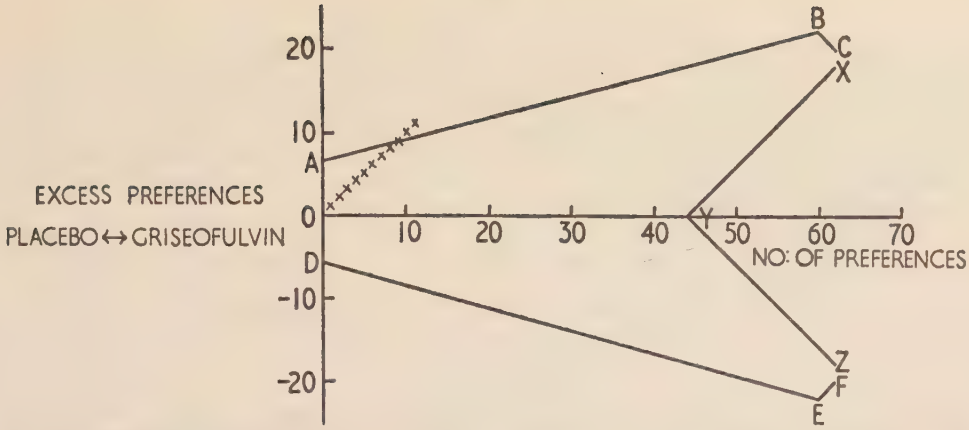

FIG. 1. Chart for sequential analysis of results. Horizontal scale records the number of pairs showing preference; vertical scale records the number of preferences for griseofulvin minus the number of preferences for the placebo. Path moves one unit "north-east" when treated member of a pair is improved, and one unit "south-east" when control member is improved.
A path reaching boundary ABC indicates a significant (P 0.05) advantage to griseofulvin; one reaching DEF would point to a significant advantage to the dummy treatment; while a path reaching XYZ would show that there was no real difference between griseofulvin and the dummy.
(From Lancet 1960 i, 1141, by kind permission of the Editor)

improvement." The sequential method, described by Armitage (1958), enables a significant trend to be detected at an early stage, at which point the entry of new patients into the trial can be stopped. Treated and control patients were grouped in pairs, and only pairs showing preference were plotted on a prepared chart (Fig. 1). A result was recorded when either the treated or the control member was improved and the other was not; if both were improved or both not improved, no action was taken. When only the treated member of the pair was improved, a cross was plotted one unit "north-east" of the origin at O. Had there been the opposite result in a pair—the control member improved and the treated not improved—the next cross would have been plotted one unit "south-east" of the preceding one. The boundary lines are drawn in such a way that, if the plotted points reach the line ABC, the treated group is showing an advantage over the control group significant at the 5% level so that the intake of new patients in the trial could be stopped. In our trial the plotted points fell on a straight line which crossed line ABC shown in Fig. 1. Though the intake of patients could, in fact, have ceased after plotting the results for the first 9 pairs showing preference, in order to be cautious the intake was extended beyond this number. When entry into the trial was eventually stopped, 76 patients had been registered and were receiving either griseofulvin or placebo tablets. It was decided to allow every patient to complete 4 months in the trial before comparing results in the treated and control groups.

RESULTS AT FOUR MONTHS

Of the 76 patients, 72 had infection with *Trichophyton rubrum* (Table III). Other data concerning these patients is stated in the detailed publication (Russell et al, 1960).

Organism	*Number of patients*
Trichophyton rubrum	70
T. rubrum and *Trichophyton mentagrophytes* var. *interdigitale* ...	1
T. rubrum and *Epidermophyton floccosum*	1
T. mentagrophyton var. *interdigitale*	3
E. floccosum	1

TABLE III

Fungi responsible for disease in 76 patients who completed 4 months in the trial.

During the early months of the trial, 12 patients (8 in the control and 4 in the treated group) left the trial for various reasons. Of the remaining 64 patients, 33 received active medication, and at the end of 4 months only 2 of them had failed to show definite clinical improvement, but these later did so (Table IV). Of 31 patients receiving placebo tablets, only 1 showed improvement after 4 months in the trial, the improvement being confined to the finger-nails. Patients on control treatment all subsequently improved when assessed 2 months after changing to griseofulvin therapy.

64 Patients	TREATED	33	IMPROVED	29
	CONTROLS	31	IMPROVED	1

(76 patients started the trial — 12 left)

TABLE IV

Double blind trial — results at four months.

Results at Nine Months

Clinical improvement and subsequent clearing of the lesions occurred first on the skin of the hands or other sites excluding the feet, and later on the feet. Nail dystrophy improved more rapidly on the fingers than on the toes.

The clinical and mycological state of the nails of those patients who have now had 9 months of active treatment is shown in Table V. At this stage 27 of 33 patients with finger-nail infection have grown normal nails, whereas only 7 of 29 patients with toe-nail lesions have done so in the same period. Mycological improvement appears to lag

		CLINICAL	*MYCOLOGICAL* (Direct Exam.)	
FINGER NAILS	33 patients	Normal 27	Negative	16
			Not known	7
			Positive	4
		Dystrophic 6	Positive	
TOE NAILS	36 patients	Normal 7	Negative	5
			Positive	2
		Dystrophic 29	Positive	22
			Negative	7

TABLE V

State of nails nine months after starting treatment.

behind clinical improvement, the difficulty in clinically assessing the end stage of cure in toe-nails is illustrated by the absence of fungus microscopically in 7 patients with dystrophic toe-nails (Table V). Examination of the toe-webs yielded fungus in 19 patients in whom clinical abnormality at those sites was either absent or confined to minimal scaling, and who had been under treatment with griseofulvin for 6 months or longer.

Toxic and Side Effects

The toxicity of griseofulvin was in question for a time following the observation by Paget and Walpole (1958) that it appeared to possess a colchicine-like action on mitoses when injected intravenously in large doses into animals. Up to the present time, oral administration in man has given no evidence that significant side effects occur, though no controlled studies appear to have been performed prior to this investigation.

Symptom or sign	Patients receiving griseofulvin (37)	Patients receiving inert tablets (39)
Headache	3	1
Dyspepsia	1	1
Abdominal discomfort	1	2
Vomiting, nausea	0	2
Diarrhoea	1	2
Ulcers in mouth	0	1
Cramps in limbs	2	0
Flushing of face	0	1
Drowsiness	0	1
Fever	1	0
Lassitude	1	0
Nails more brittle	0	1
Rashes or itching	3	4

FIGURE 2

Side-effects up to 16 weeks in double-blind trial.

During the "double blind" trial just described, the opportunity was taken to establish the incidence of side effects in the group of patients receiving active treatment, and to compare these with those found in the control group who were receiving only the inert tablets. In Fig. 2 can be seen the striking similarity of side effects in the control and actively treated groups. Without the double blind trial the impression would have been gained that 13 out of 37 patients (35%) receiving 1.5 gm. daily of griseofulvin developed subjective or objective signs and symptoms attributable to the drug. This is not to say that griseofulvin may not cause headache and intestinal upsets, but one cannot disregard the fact that the latter, e.g. abdominal discomfort, nausea, vomiting and diarrhoea, were much more common in the control group.

There was a history of a rash developing in 7 of the patients, 3 of them were receiving griseofulvin and 4 the inert tablets. In the group of the 4 control patients the rashes developed in the intervals between the routine clinic visits and therefore were not examined by either authors. The first patient, who was receiving active treatment, to develop a rash did so on the exposed parts of the skin, suggesting a photosensitivity eruption but unfortunately he defaulted and we have, therefore, no knowledge of the subsequent progress of the rash.

The second patient received the inert tablets for 4 months, and one week after starting active treatment he developed a morbilliform eruption on the arms and back of the neck, which increased in severity without further spread for 48 hours, and then faded rapidly, over a total period of 5 days. The griseofulvin was stopped, there being no recurrence of the rash on restarting the drug. About the same time one of us happened to see a man who was receiving 2 gm. daily of griseofulvin for the treatment of a long-standing *T. rubrum* infection of the skin and nails. He developed a rash which was essentially an erythematous macular eruption, and was widespread over the skin of the trunk and limbs. The drug was not stopped but reduced from 2 to 1 gm. daily.

As previously mentioned, the trial was started with a group of 12 patients who were observed during a period of 4 weeks in hospital. The following investigations were carried out during that time, initially during the first week while the patients were receiving the inert tablets and again at the end of the third week of active treatment—full blood count, blood urea estimation, and liver function tests (thymol turbidity and serum bilirubin); the urine was examined for the presence of albumin, cells and casts, and the faeces were cultured for the assessment of the bacterial and yeast flora. There was no significant difference between the results at the beginning and at the end of the 4 week period. The only exception to this was that the monocyte counts of some

of these patients appeared to rise throughout the 4 week trial period. As a result it was decided to continue to estimate the differential white blood count at fortnightly, and latterly monthly, intervals in all those patients subsequently included in the full trial.

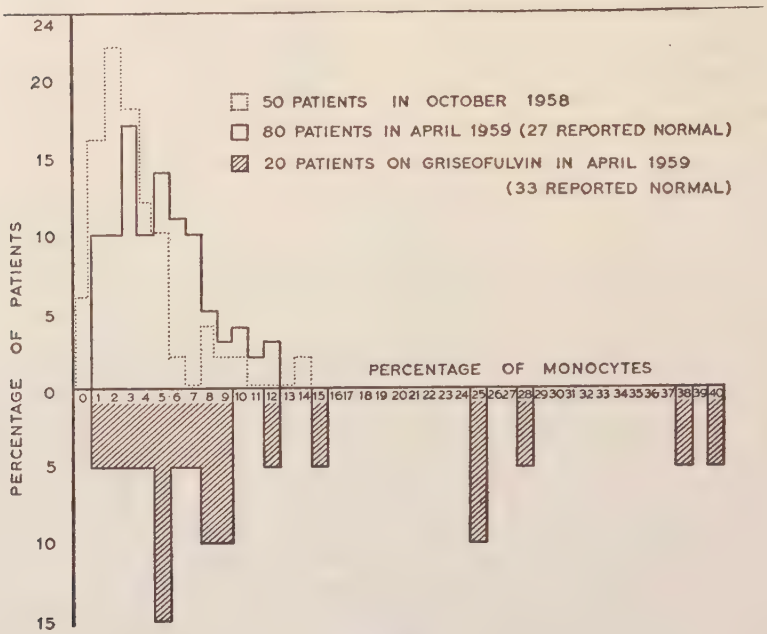

FIGURE 3

During the first half of the trial, the impression gained was that there was a relative increase in the monocyte counts in those patients receiving griseofulvin in comparison with a random selection of patients sent for routine blood counts and also from a similar group six months before in October 1958 (Fig. 3). It appeared, therefore, that there was some evidence to suggest that a raised monocyte count was occurring in those patients included in the trial.

Number of patients receiving griseofulvin	37
Number of patients receiving griseofulvin with counts above 500 per c.mm.	24
Number of patients receiving griseofulvin with counts above 1,000 per c.mm.	7
Number of patients receiving inert tablet	39
Number of patients receiving inert tablet with counts above 500 per c.mm.	26
Number of patients receiving inert tablet with counts above 1,000 per c.mm.	5

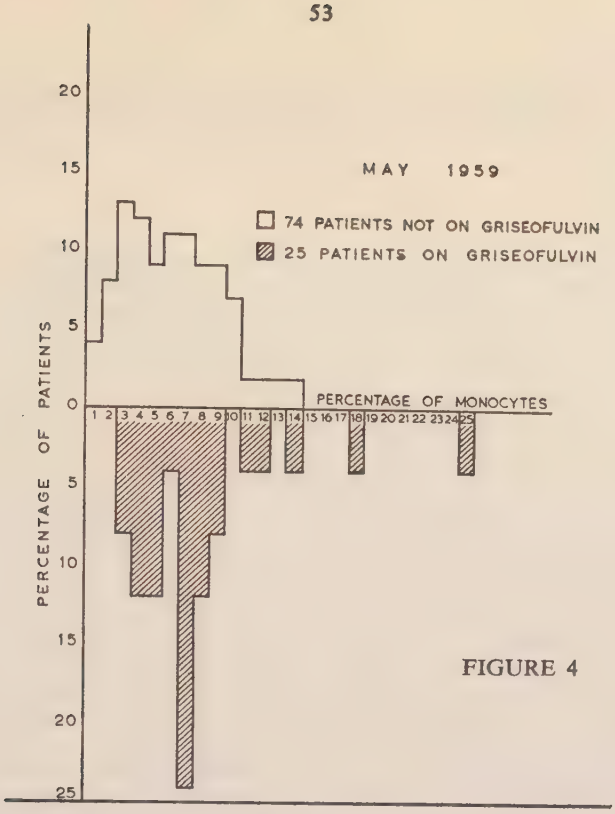

FIGURE 4

In Fig. 4, however, which was made at the end of the double blind trial, we can see that in 24 of the 37 patients receiving griseofulvin, monocyte counts above 500 per cu.mm. occurred at one time or other, and did so also in 26 of the 39 patients receiving the inert tablets. Counts above 1,000 per cu.mm. occurred in 7 of the patients receiving griseofulvin (maxima 1,880, 1,740, 1,580, 1,430 and 1,140 per cu.mm.) compared with 5 receiving inert tablets (maxima 2,110, 1,440, 1,280, 1,170 and 1,100 per cu.mm.).

SUMMARY

The results of this trial confirm the satisfactory effect of griseofulvin in the treatment of *Trichophyton rubrum* infection of the skin and nails. It would seem, however, that the oral administration of griseofulvin as a sole method of treatment is not curative in the majority of cases. Further observation is required to establish whether it produces a final cure in any patient.

A number of side effects have been associated with the administration of griseofulvin. A controlled study such as we have described helps to put these effects into their proper perspective. At the same time it should not result in the assumption that griseofulvin has been shown to be completely free from side effects, and only time will tell whether this drug is as safe as present experience would suggest. Further research into the cytotoxic effects of the drug in the experimental animal may well be required.

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DRUG REACTIONS WITH GRISEOFULVIN

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The past two or three decades have seen remarkable changes in our pharmacopoeia and in the pharmaceutical industry. Not so many years ago, before World War II, less than 20 per cent of the preparations listed in most pharmacopoeias could be shown to have any certain pharmacological action at all. Today the equivalent figure is over 75 per cent. In other words the vast majority of the drugs we now prescribe are active agents; they do something; they have a definite effect on cells, tissues, organs, or the body as a whole, when administered in adequate dosage. As a corollary it is natural that in the pre-war period scientific publications were almost entirely concerned with evidence to prove the activity and efficacy of particular drugs; whereas, parallel with the far greater pharmacological activity of present day drugs, there is now an increased emphasis on adverse or unwanted reactions to drugs. There appears to be almost an over-eagerness to report such reactions from new drugs. There is no doubt that drug reactions have become very much more frequent, if only as a result of the use of more potent drugs; some of the other contributory factors are subsidised health services and medical insurance schemes, advertising to the profession as well as to the public, the increasing desire of patients for drugs, and their administration to ever increasing thousands of persons all over the world. It is reflected in the growth of articles, monographs, and books on the subject, and the growing research interest in diagnostic and analytic investigations of drug reactions. Yet no subject appears to abound with more paradoxes. A drug may initially be rejected as too toxic for use in man, and yet may later, with some modification, become widely employed (like sulphones in leprosy); penicillin was distinguished in its early years by its almost ridiculous absence of toxicity or harmfulness, but within a decade it holds pride of place as the greatest single cause of drug reactions, having more than a hundred deaths on its record.

Before attempting to analyse the reactions with griseofulvin, we should perhaps discuss what is meant by drug reactions and side-effects. They are rather broad, ill-defined terms, comprising all undesirable actions of the drug, whether they occur in the majority or in only a small minority of patients treated. The term drug reaction implies a distinctly adverse and abnormal or unexpected effect. Side-effects are unwanted but unavoidable associated effects of drugs. The great difficulty in assessing all drug reactions is the problem of accurate diagnosis and proof. The mechanisms by which drugs may produce abnormal reactions are known to be fairly numerous; allergy is merely one of them, and not necessarily the most frequent. *In vitro* methods for the diagnosis of drug sensitivity are virtually non-existent (with a few exceptions). Hence untoward reactions are usually attributed to drugs on the basis of the circumstantial evidence of the history, coupled with knowledge acquired from previous experience. Occasionally the conclusion may be supported by recurrence of the reaction following a test dose of the suspected drug. Hence the diagnostic criteria tend to be very loose, and I believe that "drug reaction" is a very over-diagnosed condition. A few years ago Wells, in an unpublished communication to the British Association of Dermatology in London, gave his opinion that the diagnosis was very dubious in about half of a series of cases of "drug eruption" which he had analysed. The recent controlled trial with griseofulvin, carried out by Frain-Bell and Stevenson (1960), has shown very clearly how many "reactions" can easily be wrongly attributed to the active drug.

Against this background one may look at what reactions have been reported in patients taking griseofulvin. Williams, Marten and Sarkany (1958) did not mention any in their initial paper (apart from indigestion in one and thirst in two cases), but Blank and Roth (1959) in their first series of 31 patients, noted mild gastro-intestinal distress, headache,

and one skin eruption. Since then various reactions have been reported as sporadic events. They are somewhat difficult to classify. Perhaps unwanted effects of drugs are best grouped under the headings suggested by Rosenheim (1958):

1. Overdosage
   - (a) Absolute { Immediate / Cumulative
   - (b) Relative
2. Intolerance
3. Side-effects
   - (a) Specific
   - (b) Non-specific
4. Secondary effects
5. Idiosyncrasy
6. Hypersensitivity—allergic reactions

It is not always easy to decide into which group a particular effect should be placed, and several effects may be present in the same patient.

Alternatively, but less satisfactorily, reactions from a drug may be classified under the various anatomico-physiological systems, such as gastro-intestinal, haematological, cutaneous, cardiovascular, neurological and so on. Because of the limited experience at the present time with griseofulvin, however, I have chosen to discuss the reactions to it under four headings, haematological, gastro-intestinal, cutaneous and "others."

BLOOD CHANGES

Apart from anaphylaxis the type of adverse reaction which usually causes most worry to the clinician is a depressive action on the haemopoietic system. Such an action has been searched for with almost eagle eyes in regard to griseofulvin. Blank and Roth (1959) claimed complete absence of any abnormalities in blood counts on their patients, and most American observations agree with them. In England, however, a number of changes have been recorded. Frain-Bell and Stevenson (1960) noted a monocytosis (above 500 per cu.mm.) in a rather high proportion of their patients on griseofulvin, but the use of the double blind trial method appeared to completely exonerate the drug, because the same finding occurred in an equal number of patients receiving inert tablets. Leucopenia has been seen quite frequently, although there is no certain level of white cell count as being significant leucopenia (2,000 cells per cu.mm. is the usually agreed figure). Meara (1960) when treating children with *tinea capitis* on 0.25 to 0.75 gm. daily, reported a fairly regular but temporary fall in polymorphonuclear leucocyte count after starting griseofulvin. Two children, however, showed granulocytopenia as low as 650 to 1,400 cells per cu mm., and no clinician could blame him for stopping the drug. The precise cause and meaning of these low counts is obscure, but they do not seem to indicate what is usually designated as drug agranulocytosis of allergic or toxic origin. After a short interval, administration of griseofulvin was resumed in half the original dosage without further granulocyte depression being observed. In 12 cases treated by Barlow et al. (1959) regular blood counts were performed, and 4 showed much variation in the white cell count, but none fell below 2,000 cells per cu.mm. One patient's granulocyte count fell from 8,200 to 2,200 within a few days of commencing griseofulvin; it was accompanied by headache, nausea and dizziness. These symptoms stopped when the drug was withheld and the blood picture returned to normal. On restarting therapy symptoms recurred, and the count fell again from 6,500 to 2,200 within 24 hours. Fluctuation of the cell counts persisted but the drug was stopped after ten days because of the increasing severity of other symptoms. However, after a further interval the drug was restarted with the patient under observation in hospital, and was continued for six weeks without recurrence of the symptoms or granulocytopenia. Van Breuseghem (1960) noted monocytosis, leucopenia, and anaemia with punctate basophilia of red blood cells. Sarkany and his colleagues (personal communication 1960) have

studied this problem and believe that the variations in granulocyte counts (even below 1,000 cells per cu.mm.) are not necessarily a toxic effect or even related to the drug therapy.

At the present time, therefore, one may say that griseofulvin in the normal adult dosage of 1 gm. per day has produced no serious adverse reactions on the blood-forming tissues, and frequent examinations of the peripheral blood are not indicated as a routine measure.

GASTRO-INTESTINAL EFFECTS

When patients are taking a drug by mouth they have a natural and understandable tendency to attribute to the drug any digestive or abdominal symptom which they experience while on the treatment, unless there is another more obvious and acceptable explanation. This will happen whether the tablet, capsule, or medicinal mixture has any pharmacological action or not. The complaints encountered include anorexia, nausea, vomiting, various dyspeptic symptoms, and diarrhoea. While many of these symptoms may well be coincidental, it is very likely that some of them must be attributed to the drug. Two of the fifty children treated by Barlow and Mackenzie (1959) complained of nausea; in one it passed off in a few days while the drug was continued, whilst in the other the child's mother stopped the griseofulvin. One of my own patients had an attack of diarrhoea with a skin rash soon after starting the drug, and treatment was interrupted. Neither recurred when it was recommenced some months later. In another patient, however, diarrhoea necessitated cessation of treatment, and it recurred on each of several occasions that the drug was restarted. The mechanism of causation of these gastro-intestinal symptoms is obscure. They are too transient to suggest any secondary effect such as candidiasis from disturbance of the intestinal flora, as occurs with the tetracyclines and similar broad spectrum antibiotics. Nevertheless, there are already suggestions, and some evidence, that griseofulvin therapy can give rise to this troublesome complication. One should remember, however, that in the past few years the incidence of moniliasis was greatly increasing before the use of griseofulvin. The latter drug has, of course, no action on Candida albicans, but it is possible that other antibiotic substances will be found which are active against both dermatophytes and candida.

SKIN ERUPTIONS

Blank and Roth (1959) had one patient in their series with an urticarial drug eruption but remarked that there was no cross sensitivity in a patient known to be allergic to penicillin. Barlow et al. (1959) found the somewhat high incidence of five patients with rashes out of 34 treated. Morphologically they were described as erythematous, urticarial, morbilliform, or nondescript. All subsided when the drug was stopped, but in two instances the rash recurred when it was given again (in one this was repeated three times), and they may have been allergic. In the controlled trial described by Russell et al. (1960) rashes were encountered in three patients on griseofulvin, but in four on the control tablets. In the former, one was described as a papular erythema multiforme type of reaction; it cleared when the drug was stopped, and did not recur when therapy was recommenced two weeks later. The other was a widespread red papular eruption, which cleared up in spite of the drug being continued. Similar cases including morbilliform eruptions have been seen by Wells and Maibach (personal communications), and several others. It would seem evident at the present time that the majority of patients who develop rashes can continue taking griseofulvin, or take it again after an interval. This does not imply that the eruptions are not caused by the drug, but merely that the reaction is probably not a specific allergic one. Goldblatt (1960) reported an instance of severe urticaria and angioneurotic oedema. His patient was a man of 30 years who was given treatment with intravenous cyanocobalamin for lichen planus, and griseofulvin

for a ringworm infection in a dosage of 2 gms. per day for a week before the complication occurred. The drug was stopped and corticosteroid therapy given with relief. The evidence, however, was no more than circumstantial to implicate griseofulvin although the author stated that attempts were being made to elucidate the case with test procedures.

A somewhat unexpected effect has recently been experienced. Hall-Smith (personal communication, 1960) has seen two patients in Brighton, Sussex with light sensitivity eruptions which developed for the first time on griseofulvin, and which rapidly subsided when the drug was stopped. Other similar cases have apparently been seen in the United States (Witten, personal communication, 1960), and it is possible that photosensitivity will prove to be a genuine hazard.

OTHER EFFECTS

A. *Spermatogenesis*

In view of Paget and Walpole's (1958) statement that griseofulvin was capable of arresting the development of cells of the testis, there was a good deal of anxiety about the effect on spermatogenesis. Koller's (1960) experiments were not widely known when griseofulvin was being used initially for clinical trials, but one clinician had obtained evidence suggesting a depression of sperm counts (Hildick-Smith, personal communication, 1959). MacLeod and Nelson (1959) have reported on a clinical and histological study of the problem. Fourteen healthy human male volunteers were given 2 gms. of griseofulvin per day by mouth for three months. Although the spermatazoal counts in semen varied widely there was no evidence of a depression, nor of any effect on the morphology of the sperms. In eight cases histological examination of the testis was made before and during treatment, and again no abnormalities were found. This is the expected finding from the evidence of Koller (1960) that the arresting effect of griseofulvin on cells in the metaphase is a transient and short-lived one.

B. *Fever*

One of the patients of Barlow et al. (1959) who developed a rash also had pyrexia, which appeared to be a genuine example of drug fever.

C. *Headache*

Quite a number of patients receiving griseofulvin have noticed headaches, but they have rarely been bad enough to merit an interruption of therapy, except in the severe migrainous type. The headache usually passes off with continuation of treatment, especially if the dose can be lowered. Analgesics should be used to relieve the symptom. Giddiness has been reported in some patients.

D. *Miscellaneous*

General malaise and lassitude have been attributed to the drug, as have thirst, cramps and urinary symptoms. Most of these are extremely difficult to assess in relation to griseofulvin, and are not indications for cessation of treatment; but they should receive symptomatic medication.

CONCLUSIONS

It is inevitable that certain symptoms and signs will be experienced by patients receiving griseofulvin, which cannot be related to their dermatophytic infection. When they occur the clinician must make an immediate decision on whether or not to interrupt the drug therapy. In this he will be guided mostly by their severity, and the size of the dose the patient is receiving. If the symptoms are relatively trivial and not disturbing to the patient, they may be ignored. If they are worse and causing discomfort, the dose of the drug should be reduced to the minimal effective level (if that is not already being

used), and appropriate symptomatic treatment prescribed. If this is not effective, or if the symptoms are of major severity, griseofulvin treatment should be stopped until the patient recovers; but it may then be restarted after an interval, initially on a much lower dosage, and increasing it cautiously until the minimal effective level is reached.

If, however, abnormal signs appear, whether in the haematological, renal, or other symptoms, it is usually wise to stop treatment and to investigate the abnormality. Provided that the abnormal signs rapidly clear up or can be shown to be unrelated to the drug, the griseofulvin can again be administered, but with caution, having the patient under close observation (in hospital, if necessary), and in a low dose such as 0.25 gm. per day before gradually increasing it up to the effective level.

It is probably safe to conclude that so far no adverse or side-effect of the drug has been encountered, which should be considered as an absolute contra-indication to further griseofulvin therapy. To avoid reactions, the minimal effective dose should be used. If minor symptoms occur they should be treated. In the presence of more severe symptoms or abnormal signs, the physician should not regard them as the red light, but as an amber, and may proceed with caution.

SUMMARY

Unexpected reactions and side-effects are almost inevitable concomitants of any pharmacologically active drug. They may be numerous and diverse, and the mechanisms involved in their production are equally varied. There is now a heightened awareness of adverse reactions to drugs, and not infrequently symptoms and signs are wrongly attributed to specific drug therapy.

Many unwanted effects have been reported with the use of griseofulvin, but none has been disastrous or even of major severity. Minor troublesome symptoms, which may occur, are headache, anorexia, nausea, vomiting, and diarrhoea. Rarely do they necessitate interruption of therapy. Irregular haematological changes include monocytosis, granulocytopenia, and punctate basophilia, but none of them has been severe enough to cause alarm. Cutaneous eruptions have been erythematous, papular, urticarial, morbilliform and of erythema multiforme type. Photosensitivity may also occur. Only in exceptional instances has the reaction recurred on repeated exposure to the drug. Occasional symptoms are malaise, headache, giddiness, thirst and fever. No appreciable depression of spermatogenesis is found. There is slight evidence that candidiasis may be a possible complicaton.

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TINEA CAPITIS TREATED WITH GRISEOFULVIN

R. H. Meara

Since December 1958 all patients admitted to the Ringworm Unit at the Goldie Leigh Hospital, have been treated with oral griseofulvin. The results of treatment of the first group of patients have recently been published (Meara, 1960) and since then a few more patients have been treated and the first group followed-up for a further period. No controls were used in this clinical trial as it was considered that the natural history of the disease was known well enough to act as an historical control—and there were ethical difficulties about withholding treatment or giving treatment known to be ineffective.

DETAILS OF TREATMENT

The first 22 children were treated with a daily dose of 0.75 gm. griseofulvin for older children and 0.375 gm. daily in children of four years or under. Subsequently children over four were given 0.375 gm. daily, younger children 0.25 gm. daily. This dose was approximately 15 mg. per kilo. Treatment was continued for 3 to 5 weeks, depending on the rapidity with which non-infected hair grew. No local treatment was given apart from daily scalp shampoos and the removal of infected hair by clipping or shaving when sufficient normal growth had appeared proximally to make this possible. The scalp was not otherwise shaved or clipped.

RESULTS OF TREATMENT

Microsporum Audouini. Twenty-six patients have been treated. Three needed second courses of treatment—two because some infected hairs persisted—and one because after clinical cure, further patches of ringworm appeared at sites different from the original ones. No evidence of infection of the skin was found at these sites before fluorescent hairs were seen.

Microsporum Canis. Ten patients were treated, only one needing a second course of treatment because of the persistence of infected stumps.

Other Varieties. One case of Trichophyton discoides infection—a gross kerion—appeared to subside very rapidly with this treatment. One example of Trichophyton rubrum infection of the scalp showing as an inflammatory endothrix infection responded satisfactorily. Only one case of Trichophyton violaceum infection was treated. She was clinically cured after one course of treatment, but three weeks later fresh lesions appeared. The scalp was affected for some weeks before infected hairs could be found. Three months after a second course of treatment, no evidence of infection was found.

LATE RESULTS

All the children have been followed-up for at least three months after they were considered cured and discharged from hospital. No late relapses have as yet been observed. It seems unlikely that relapses will occur after this period, at least in fluorescent ringworm where a few persistent stumps are easily seen.

SIDE-EFFECTS

No side-effects were noted except for a decreased polymorphonuclear leucocyte count in two children. Haemoglobin estimations and differential white cell counts were carried

out routinely before, during and some days after stopping griseofulvin therapy. Considerable variations in the total polymorph count occurred but there appeared to be a fairly regular fall during the first week of treatment, rising again after a few days. The total polymorphonuclear leucocyte count fell in one child from 4,350 cells per cu.mm. before treatment to 650 per cu.mm.; and in another from 3,250 per cu.mm. to 1,400 per cu.mm. Both children were having second courses of treatment and the polymorphonuclear leucocyte count rose to normal within a few days of stopping treatment.

DOSAGE AND DURATION

The lower dose mentioned, 0.375 gm. daily for children over 4 and 0.25 gm. daily for younger children, appeared to be just as effective as the larger dose given to the first 22 children. As it happens, all the relapses occurred in children having the larger dose.

One child with *Microsporum audouini* infection was deliberately given treatment for only 7 days. After 3 weeks normal hair growth was visible and the child was cured, showing no evidence of infection five months later. A child treated for 10 days, relapsed 4 weeks after stopping treatment, having been apparently cured. No further short courses of treatment were given. It is probably more reasonable to give treatment for at least 3 weeks than to attempt cure by giving a single very large dose of the antibiotic. The time taken to cure depends very largely on the rate of growth of the hair.

LOCAL TREATMENT

All the children were in-patients and under close observation. Scalp shampoos were given daily. The incidence of relapses, although low, might be reduced by the application of a local "fungicide," but this has yet to be shown. Local treatment is probably more necessary in children treated as out-patients. The persistence of infective material on scalps where fluorescence has disappeared may be another indication for using full local measures (Beare, 1959).

SUMMARY

Thirty-nine children with *tinea capitis* due to different types of fungus were treated with oral griseofulvin. All were cured, although in a few cases second courses of treatment were necessary.

No local treatment was used apart from daily shampoos and the removal of infected hairs when sufficient normal hair had grown to make this possible.

ACKNOWLEDGEMENTS:

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THE PLACE OF GRISEOFULVIN IN THE MANAGEMENT OF TINEA CAPITIS

J. Martin Beare

From 18 months' use of griseofulvin in the treatment of tinea capitis there have emerged two facts: —

(a) griseofulvin alone is insufficient,

(b) griseofulvin combined with recognised procedures in Public Health management makes feasible the complete elimination of most varieties of tinea capitis from our community.

Microsporum Canis

Our original series, consisting of 19 cases treated in the period November 1958 to March 1959, has already been reported in detail (Beare and Mackenzie, 1959). The main conclusions reached were: —

(a) Fungal invasion of hair is stopped abruptly soon after the drug is given and new areas of infection do not appear. Debris, probably intrafollicular, remains infected and the organism can be recovered readily for long periods after treatment is started.

(b) Wood's light fluorescence usually disappears in one to six weeks (average three weeks).

(c) With *M. canis* infections fluorescent tips are only exceptionally found.

In the above series the majority of patients did not receive any form of local treatment until about half-way through the trial, when it became apparent that oral griseofulvin therapy alone failed to eradicate infection satisfactorily; then mothers were told to scrub and wash the child's scalp twice daily after the initial three to four days' treatment, and local fungicidal preparations were regularly applied. A typical child from this series is depicted in Fig. 1, and it will be noted that mycological cultures were still positive for 16 weeks after treatment with griseofulvin was started.

Marion R. (4 yrs.) Duration 4 weeks.	Tinea capitis et corporis (M. canis) Non-inflammatory.								
Weeks	0	2	4	6	8	10	12	14	16
Attendances	xx	x x	x	x x	x x	x	x	x	
Griseofulvin	ooo	ooooo	ooooo	ooooo	ooooo	ooooo			
Wood's light	++ ++	+ + +	—	— —	— —	—	—	—	
Cultures	++		+	+ +	+ +	+	—	+	

FIGURE 1

There was also in this series one unusual patient who was unique in several fortunate ways. In the first place he had one week's treatment, followed, owing to an oversight, by one week without treatment, then treatment was started again and regular examinations were continued. Secondly, the hyphal invasion of this boy's hair was insufficient (either because of minimal invasion by the fungus or because of the unusual robustness of the boy's hairs) to cause infected hairs to break. The result was that we obtained long hairs for examination, many of which showed in continuity bands of fungus invasion and bands of fluorescence illustrating the effect of the drug. Fig. 2a shows a hair from

the child photographed in ordinary light and Fig. 2b shows the same hair photographed with a double exposure of both ultra-violet light and visible light; in the latter two fluorescent bands with a non-fluorescent band intervening can be clearly seen; the non-fluorescent part represents the period of treatment with griseofulvin.

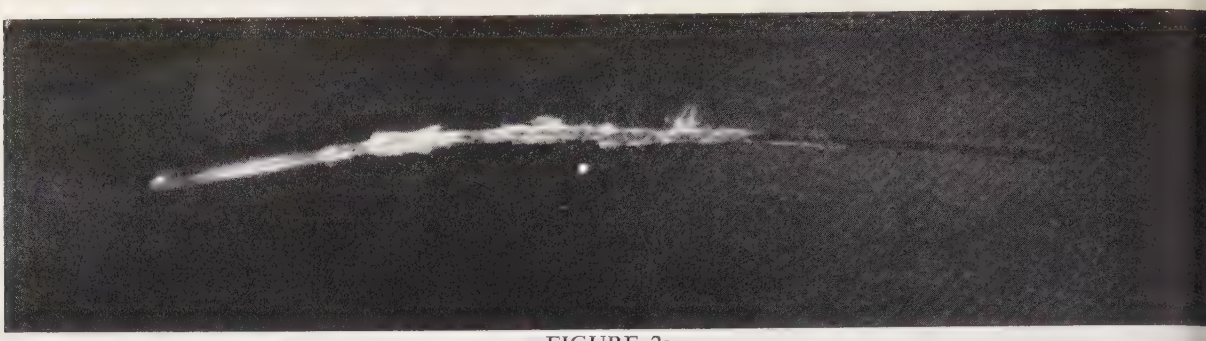

FIGURE 2a
Control

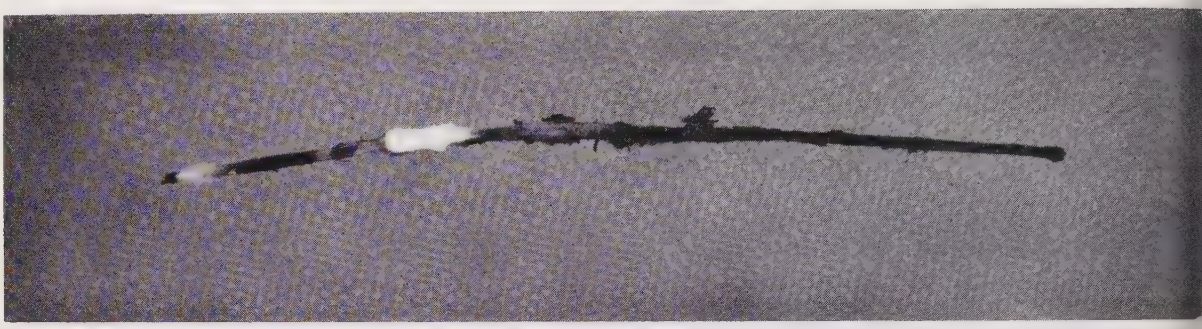

FIGURE 2b
Fluorescence — Ultra Violet and visible illumination

Since these 19 cases were seen and studied, a further seven cases of *M. canis* scalp infection have been treated with griseofulvin (up to November 1959). One, a baby aged three weeks, was unable to tolerate griseofulvin syrup and had to be omitted from the trial, leaving six children aged between four and 10 years. One of these infections was of the rare non-fluorescent variety. These six children were given full local treatment

Case	Age (yrs)	Duration before treatment (weeks)	Last positive obtained (weeks after starting treatment)			Duration of treatment (weeks)
			Wood's light	Microscopy	Culture	
John S.	8	2	neg. always	1	5	8
Daniel R. ..	10	3	9	9	8	12
Patrick McC. ..	4	3	3	1	1	4
Hugh G. ..	5	2	1	2	3	5
Gerard F. ..	6	½	2	2	5	11½
Alan B.	4	½	1	7	3	15

FIGURE 3
New cases of M. canis scalp infection treated with griseofulvin and all local measures

as well as griseofulvin, and the results are shown in Fig. 3. Though the numbers are small it is of some interest that, with one exception, all were mycologically negative by six weeks. The exception referred to is depicted in Fig. 4. The child was, among other things, shaved by our Nursing Sister every week at the clinic, yet he could not be regarded as cured from any point of view, even including Wood's light fluorescence, until the 10th week.

Daniel R. (10 yrs.) Duration 3 wks.	Tinea capitis (M. canis)																					
Weeks	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Attendances	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x				x		x
Griseofulvin	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
Wood's Light	+	+	+	+	+	+	+	+	+	+	—	—	—									
Microscopic	+	+	+	+	+	+	+	—	—	+	—	—	—			—				—		—
Cultures	+	+	+	+	+	+	+	+	+	—	—	—	—		—	—				—		—

FIGURE 4

It will be seen, therefore, that, even with shaving and the application of strong fungicides locally, it is not always possible to get *M. canis* infections of the scalp clear in reasonably short periods; and, since we know that *M. canis* infections clear spontaneously within three to six months, one can hardly argue that the duration of this infection is appreciably shortened by the giving of griseofulvin. The advantage of the treatment for the child lies mainly in the fact that no new areas of infection develop. The disadvantage, and it is considerable, lies in the fact that Wood's light fluorescence frequently disappears early and one has to rely entirely on mycology to determine when the child is free of infection.

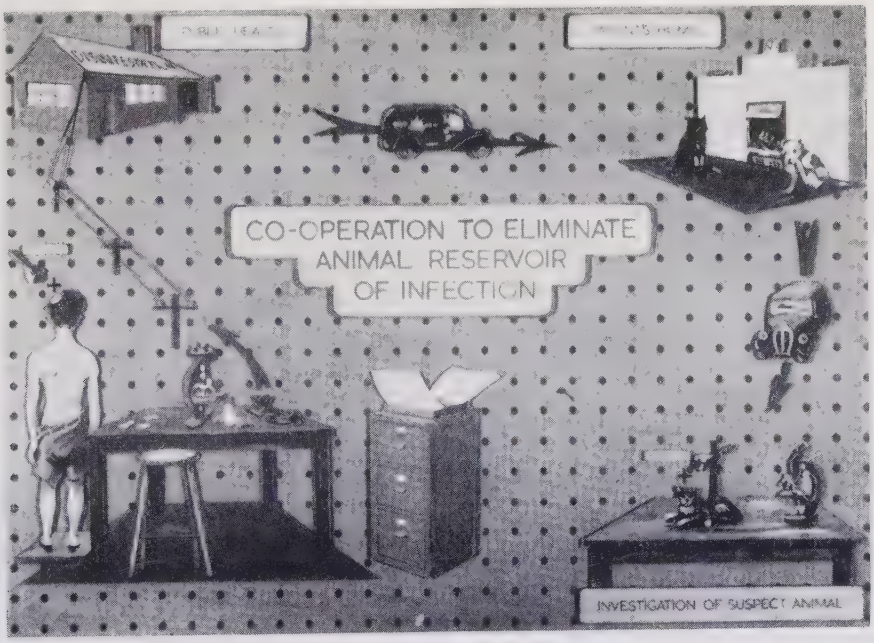

FIGURE 5

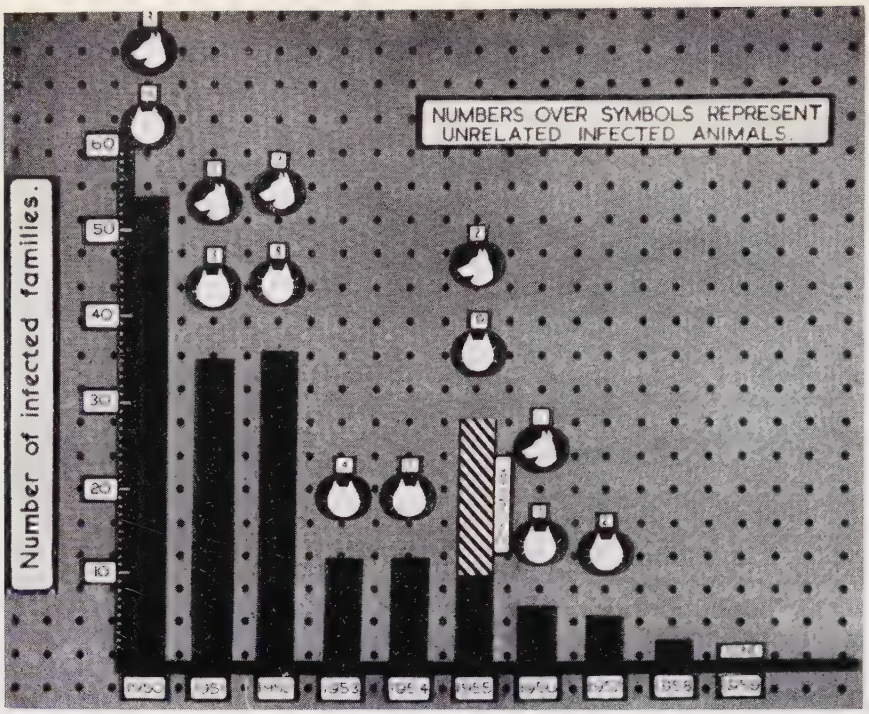

FIGURE 6

I am grateful to Dr. La Touche for the loan of Fig. 5 which shows clearly the correct practical method for dealing with outbreaks of *M. canis* infection in a community; and for Fig. 6, showing the success of such methods (without the use of griseofulvin) as practised by him in the Leeds area.

One may justifiably comment that the elimination of the reservoir of infection in the cat population is more important than the treatment of the individual child with griseofulvin. This is mainly because—in practice—a child suffering from ringworm due to *M. canis* does not spread infection, but the cat does.

Trichophyton Verrucosum

Fig. 7 gives the details of 10 children with scalp infection due to *T. verrucosum* seen in the twelve-month period from November 1958, and also of three adult scalp infections seen during the same period. None of the cases developed new lesions while on treatment. All these were treated with griseofulvin for what we thought was an adequate period. All had adequate follow-up and all had the full advantages of local treatment with starch poulticing, manual removal of hairs, etc.

The 10 children have been grouped in this table in order of duration of infection before treatment, since, if griseofulvin can be given early in inflammatory ringworm, then one might hope to abort the infection; but, on the other hand, if the drug is given late then one cannot hope for much. Six of the children (and two of the adults) produced positive mycological cultures at the initial examination only, but these six cases are scattered up and down the table and this result seems to bear no relationship to the duration of infection before treatment.

Case	Age (yrs)	Duration before treatment (weeks)	Last positive obtained (weeks after starting treatment)		Duration of treatment (weeks)
			Microscopy	Culture	
Paul W.	9	1		8	8
John S.	15	1		initial only	3*
Allen McM. ..	4	2	initial only	initial only	5
Alan McD. ..	11	2		initial only	7*
John McN. ..	6	3	initial only	3	5**
Peter O'H. ..	6	3		3	5
Edna T.	7	4		initial only	8
Nigel E.	4	4	2	initial only	7
Andrew B. ..	8	4	8	3	8
Peter K. ..	6	6	2	initial only	4
Adults					
O'B.	61	3		initial only	7
McC.	44	3	7	initial only	10*
D.	27	3		4	4

* 500 mgm. t.i.d.
All other patients received 250 mgm. t.i.d.
** Developed "id" reaction on day after starting Griseofulvin. This reaction lasted 3 weeks.

FIGURE 7
Tinea capitis (T. verrucosum) treated with griseofulvin

It is difficult to understand why one of these cases (Paul W.) should still be culturally positive after no less than eight weeks, but the importance of this in cattle ringworm is doubtful. The violence of the inflammatory reaction in this type of ringworm will loosen hairs early in the child's illness, and presumably griseofulvin cannot then enter these particular hairs. Yet such a hair could readily remain undetected for long periods and the chance finding and subsequent culturing of that hair at eight weeks (or later) is unimportant. Photo A shows Paul W. at eight weeks and clearly from the clinical point of view his condition is quite satisfactory. The mycological follow-up of cases of *T. verrucosum* infection is then of doubtful value. The behaviour of the three adults was in no way different from that of the children.

Only one "id" reaction was seen in the 13 cases. This developed the day after griseofulvin was started in a child who had had the infection for three weeks. Contrary to opinion expressed elsewhere, I do not think that griseofulvin tends to increase the number of "id" reactions in kerionic ringworm.

It is my impression, and I can think of no way of proving this statistically, that most cases of *T. verrucosum* infection settle more quickly on griseofulvin than they otherwise would. This is illustrated in Photo B1 and B2 (Peter K.) taken after three and 10 days' treatment respectively. In this particular case (the only one of non-scalp ringworm discussed in the paper) the location of infection in the eyebrow and eyelash area made local treatment impractical so that virtually the only treatment given was griseofulvin. On the 10th day microscopically positive hairs were found, but nothing grew on culture. Thereafter he was mycologically negative from both points of view.

On the other hand, Photo C1 and C2 (Mrs. McC.) illustrate one of the adult patients who had full local therapy with starch poultices, etc. and whose infection did not appear to be influenced by treatment. Although culture positive only on the initial examination, she had microscopically positive hairs for seven weeks. From the clinical point of view the infection ran the usual course familiar to us before the introduction of griseofulvin and lasted 10 weeks.

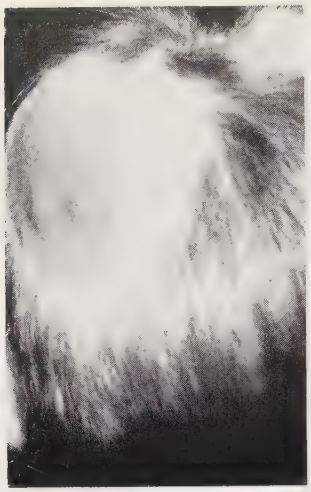

PHOTO A

Paul W. (*T. verrucosum*) 8 weeks after the beginning of treatment. Clinically well but still mycologically positive.

PHOTO B1

Peter K. (*T. verrucosum*) three days after starting griseofulvin.

PHOTO B2

Same child 10 days after starting griseofulvin and illustrating dramatic response to treatment.

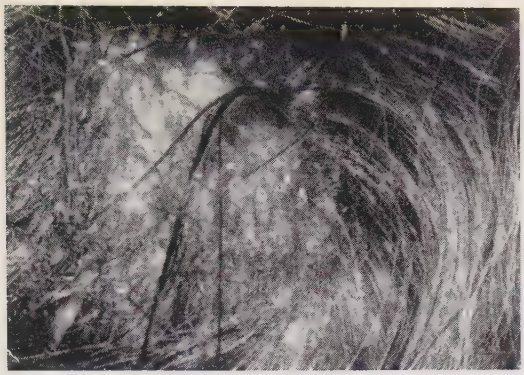

PHOTO C1 (*left*)

Mrs. McC. the day treatment began. (*T. verrucosum*)

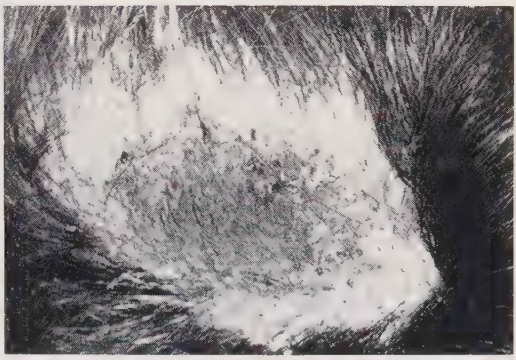

PHOTO C2 (*right*)

Same patient after 10 weeks' treatment and illustrating little benefit from griseofulvin.

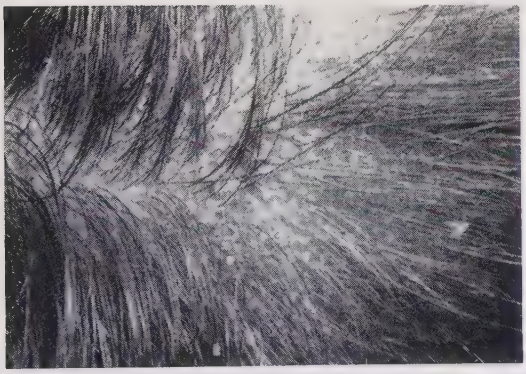

PHOTO D (*left*)

Tinea capitis (*T. sulphureum*) in a child aged nine years showing no response after 11 weeks' treatment.

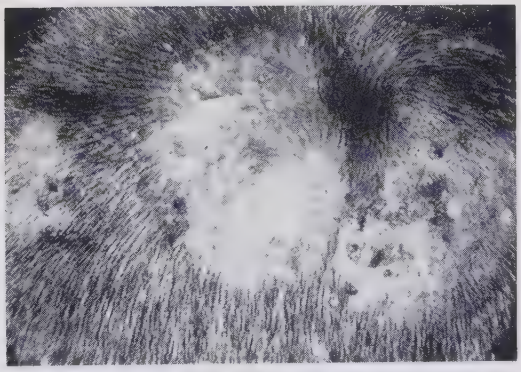

PHOTO E (*right*)

Patrick W. (aged 10 years)—inflammatory *T. sulphureum* infection of the scalp (see Table 9).

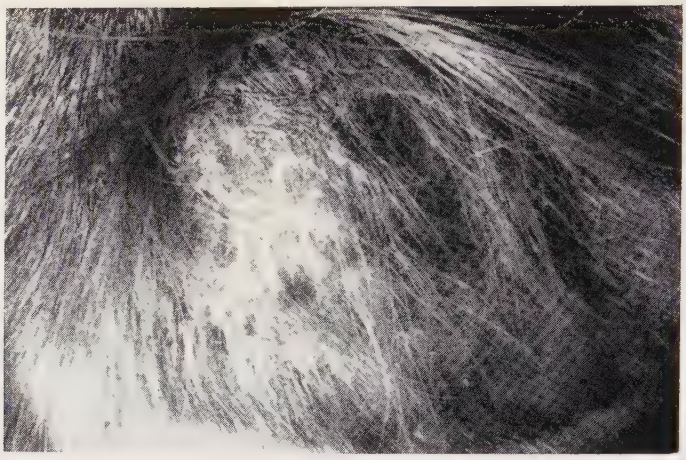

PHOTO F

John B. (aged five years)—inflammatory *T. sulphureum* infection of the scalp (see Table 9).

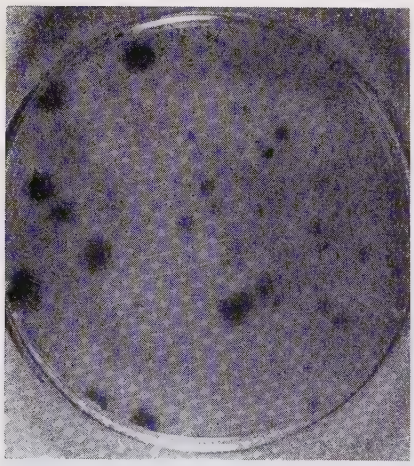

PHOTO G

Result of placing a hairbrush used by a child ostensibly infected on to a culture plate. There are 12 colonies of *T. sulphureum* in the illustration.

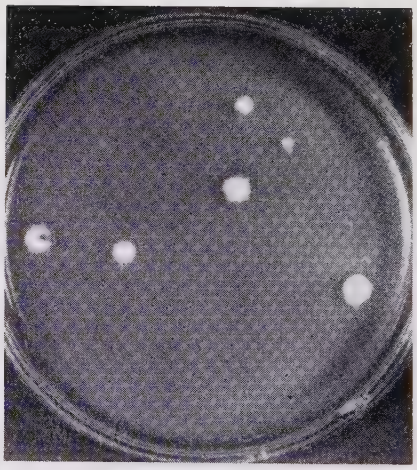

PHOTO H

Culture plate exposed during bedmaking near a bed occupied by a child with *T. sulphureum* infection. There are six colonies of *T. sulphureum* present.

Case	Age (yrs)	Duration before treatment (weeks)	Last positive obtained (weeks after starting treatment)		Duration of treatment (weeks)
			Microscopy	Culture	
Mary V.	25	5	5	5	9
Ian T.	2	2	initial only	9*	7

* Negative 3rd—8th week
Dosage: 250 mgm. daily

FIGURE 8
Tinea capitis (T. mentagrophytes) treated with griseofulvin

Trichophyton Mentagrophytes

Fig. 8 illustrates the two cases of *T. mentagrophytes* infection treated. They did not appear to behave in any manner different to the *T. verrucosum* infections.

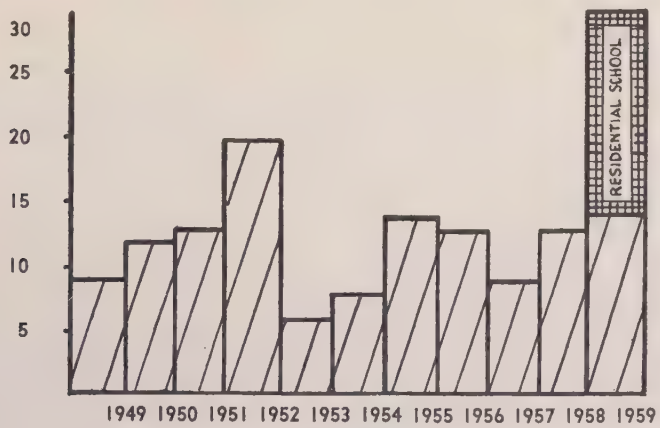

FIGURE 9
T. Sulphureum scalp infections in Belfast.

82 cases seen 1949-1955 of which 28% were inflammatory and 12% of the total severely so. In 3 the kerion reaction was delayed for several weeks.

Trichophyton Tonsurans Var. Sulphureum

As illustrated in Fig. 9, we have been troubled by this type of fungus infection in Belfast for some time. Ignoring the group of residential schoolchildren, there is nothing to suggest an epidemic, but the situation was potentially dangerous. Routine treatment (often by X-ray epilation) and clinical examination of school and home contacts by the school medical and nursing services had failed to eliminate this fungus from our area.

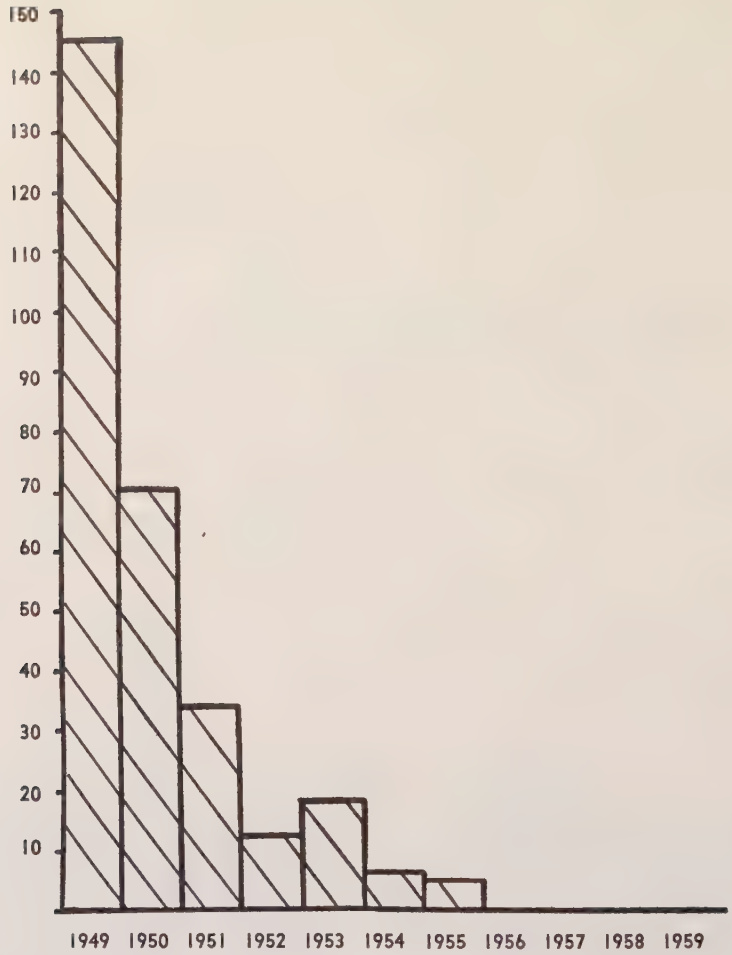

FIGURE 10

Number of M. Audouini Scalp infections in Belfast in ten-year period.

On the other hand, as illustrated in Fig. 10, we have had no cases of *Microsporum audouini* infection since 1955. It is indeed strange that the same Public Health methods carried out by the same people in the same area worked so well with *M. audouini* and failed with *T. sulphureum*. I do not believe that this decrease in the number of cases of *M. audouini* had anything to do with loss of virulence of the fungus, but this is not the time or place to elaborate on that point; suffice it to say that there are areas in the world today where the incidence of *M. audouini* remains very high. Rather I think it is due to the fact that the carrier state in *T. sulphureum* is impossible to detect without first-class mycological techniques, whereas with *M. audouini* it is easy. There are two reasons for this—(a) *T. sulphureum* infected hairs do not fluoresce under Wood's light and

(b) *tinea corporis* is just as common with *T. sulphureum* as *tinea capitis* but is unimportant with *M. audouini*. Furthermore, since one frequently cannot see any abnormality in a *T. sulphureum* carrier, or at least the abnormality is so slight that no parent is going to regard it seriously, one could not justify the use of X-ray epilation. Now the position has changed and with griseofulvin we have an acceptable method of treatment.

Inadequate attendance and/or treatment	2
Failure to re-attend after 3, 4, 9 and 11 weeks' treatment. Mycologically positive at last attendance.	4
Non-residential school children now mycologically negative.	8
Residential school children all now mycologically negative.	16

FIGURE 11

T. sulphureum scalp infections treated with griseofulvin

Our cases of *T. sulphureum* infection of the scalp fall conveniently into four groups as shown in Fig. 11. Two children had inadequate and irregular treatment and can be disregarded. Four had regular treatment for a variable time but failed to continue for as long as advised. However, it is important to note that two of these children had treatment for 9 and 11 weeks respectively but yet we failed to cure them. Photo D shows the child referred to above after 11 weeks' treatment.

Case	Age (yrs)	Duration of treatment (weeks)	Clin. assess.	Last positive obtained (weeks after starting treatment)		Remarks
				Microscopy	Culture	
John B.	5	6	4	2	2	Kerion.
Patrick W.	10	6	4		1	Kerion.
James D.	9	6	3	2	3	Mod. inflam.
Joseph K.	10	6	2	4	3	Mild inflam.
John McN.	7	7	2	2	2	Mild inflam.
Gerald McN.	9	7	3	4	3	Mod. inflam.
Marie K.	5	3	2	1	1	Mild inflam.
Ann D.	9	13	3	6	7	Mod. inflam.

FIGURE 12

Details of T. sulphureum scalp infections treated with griseofulvin (Non-residential school children).

Adequate treatment and follow-up

The group of eight children who did not belong to the residential school but who attended for an adequate length of time and follow-up is shown in Fig. 12. The results here are excellent but, curiously, all were to some extent inflammatory as exemplified in Photos E and F.

Case	Age (yrs)	Duration of treatment (weeks)	Clin. assess.	Last positive obtained (weeks after starting treatment)		Remarks
				Microscopy	Culture	
Patricia M. ..	8	9	2			4 +ve cultures at weekly intervals before treatment. All others —ve.
Carmel D. ..	9	9	1			
Maire G.	8	9	2			
Maire R.	9	9	2			3 +ve cultures at weekly intervals before treatment. All others —ve.
Stella D.	8	9	1			
Kathleen S. ..	13	9	2			1 +ve cult. before treatment. All others —ve.
Rita C.	10	9	1			
Patricia L. ..	11	7	2			
Mary C.	10	8	2		2	
Eileen B.	8	12	2	2	4	
Bridget M. ..	9	8	2		2	
Maevis McC. ..	10	8	1		1	
Kathleen T. ..	9	8	2		1	
Geraldine G. ..	9	17	3		5	
Jean H.	7	12	2	8	5	
Bridget F. ..	10	13	2		8	

FIGURE 13

Details of T. sulphureum scalp infections treated with griseofulvin
(Residential school children—all girls)

All 16 of the residential school children had non-inflammatory *tinea capitis*; indeed some of the lesions were so minute and insignificant that it was only with the help of Dr. Mackenzie's excellent mycology that we could identify these carriers of infection. The results obtained are illustrated in Fig. 13. The overall problem was that these residential school children were being adopted at intervals by foster-parents and carrying their infection into the general community of Belfast (and elsewhere). This school was our reservoir of infection comparable to the animal reservoir of infection for the zoophilic fungi. It is of interest that the infection within the school was always non-inflammatory (and known to have been present for at least nine years), whereas the majority, but not all, of the infections outside the school were inflammatory. Admirably supervised by Dr. Mackenzie, the method used to eliminate infection can be summarised as follows: —

1. Griseofulvin treatment for all children known to carry *T. sulphureum* (250 mgms. three times daily).
2. Prophylactic griseofulvin for all other children in the school (50 mgms. per child per day).
3. The autoclaving of all articles likely to be handled (total about 10,000). (See Photo G).
4. The scrubbing of all floors and the application of strong disinfectant. (See Photo H).
5. The painting of all cupboards, etc.

6. *Repeated* examinations of all articles handled by the children, of the floors, curtains, etc. in the building itself and of all children including those who, as far as we knew, were never infected.
7. An adequate follow-up period (now six months—and this will be extended).

Jean H. (7 yrs.) Tinea capitis (T. sulphureum)
Duration 4 wks.(?)
Clin. appearance at first attendance—Large scaly circular area occiput

Weeks	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Attendances	x	x	x	x	x	x	x			x	x	x	x	x	x			x
Griseofulvin	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
Microscopic		+	+	+	+	+	+			+	—	—	—	—	—			—
Cultures		+	+	+	+	+	+			—	—	—	—	—	—			—
Clin. assess.	2	2	1	1	1	0	0			0	0	0	0	0	0			0

FIGURE 14

The result was, by and large, the best which we have yet seen and the reason can only be that, as well as giving griseofulvin, all other methods to eliminate fungus from the environment were employed simultaneously (Mackenzie, et al., 1960). Fig. 14 illustrates a typical result.

SUMMARY

Ringworm being an infectious disease it should be possible to eliminate it from a community. For varying reasons in the past this was impractical. The use of griseofulvin alone will not lead to success, but the proper integration of griseofulvin therapy, local management (removal of infected debris, loose hairs, etc.), and established Public Health procedures to eliminate the source of infection, now makes it likely that complete success will prove possible. Such success may well be realised with regard to scalp ringworm before we know why it should be that certain children react with a severe inflammatory response to a particular fungus, or why others carry this fungus without producing any signs or symptoms at all, and still others, equally exposed, neither carry the fungus nor develop infection.

M. canis
1. Eliminate source of infection.
2. Local treatment.
3. Griseofulvin.

T. verrucosum
1. Local treatment.
2. Griseofulvin.
3. Eliminate source of infection.

T. sulphureum
1. Griseofulvin.
2. Eliminate source of infection.
3. Local treatment.

FIGURE 15

Summary of order of importance of various aspects of management of tinea capitis due to three common fungi.

Elimination of the source of infection, local management and griseofulvin therapy vary in their relative importance, depending on the fungus responsible. The order of importance is illustrated in Fig. 15.

With *M. canis* infections the essential thing is the detection and removal of the infected cat(s).

With infections due to *T. verrucosum* local treatment consisting of poulticing and the manual removal of infected hairs takes pride of place, though in many cases, but not all, the duration and severity of the kerionic reaction can be decreased with griseofulvin therapy. The elimination of the animal reservoir is, at the moment, impractical but, were it not for the expense involved, possible.

Without griseofulvin the elimination of *T. sulphureum* infections is impossible, but detection of non-clinical carriers and the removal of spores from the environment are just as important as griseofulvin therapy. There is probably a place for prophylactic griseofulvin in the management of institutional outbreaks, if not indeed within the family.

ACKNOWLEDGMENTS

I wish to thank my colleague Dr. Donald Mackenzie for the loan of Photos G and H and for the invaluable help which he has given me during the past year. Dr. Mackenzie's appointment was made possible by a generous grant from the Medical Education and Research Committee of the Northern Ireland Hospitals Authority. Mr. Ronald Woods prepared all the photographs, except Fig. 2a and 2b which were kindly prepared for me by the Photographic Section of Glaxo Laboratories Limited. Miss May Willan prepared all tables, except 2 and 3 which were kindly loaned to me by Dr. La Touche. Dr. Ivan McCaw allowed me to use several of his cases and greatly helped by discussing various problems. Dr. Leonard Walby provided, as always, ideal co-operation between dermatological and school clinics. Finally, I must record the help and advice obtained from Messrs. Glaxo Laboratories Limited and for generous supplies of griseofulvin which they so readily provided.

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TREATMENT OF FAVUS WITH GRISEOFULVIN

R. Degos, E. Rivalier and P. Lefort

Since the beginning of 1959 all patients with dry ringworm of the scalp, in the St. Louis Hospital in Paris, have been treated with griseofulvin. There were 180 patients, including 42 favus, most of these being North African. Though some recurrences requiring a second course of treatment have been observed among children with tinea tonsurans, there have been so far no failures, even partial, among children with favus of the scalp. Contrary to what might be imagined considering the peculiarly resistant and tenacious nature of this type of ringworm, favus is the most remarkable triumph of griseofulvin. Moreover, our clinical reports accord with the cases lately presented at the French Society of Human and Animal Mycology by Coudert, Girard and Battesti from Lyons, and by Vincent, Drouhet and Neel from Tangier.

Doses of griseofulvin orally administered have been the same for all infections. At the start of this series we gave 1 gm. daily to children aged 6 to 12 years, and 2 gm. daily to adults for 30 days, without any topical auxiliary therapy. It very quickly became evident from the microscopic examination of hairs that the early arrest of the growth of the dermatophyte would make it possible to reduce the doses without mishap. Therefore we gave the children 0.75 gm. daily, continuing with 0.5 gm. until the end of the treatment. Adults received 1 gm. a day during the whole treatment. The duration of treatment was reduced from 30 to 21 days, and the quality of results was not impaired.

As a prophylactic precaution we prescribed local care; weekly shaving, frequent shampoos and anointment with salicylated iodine alcohol. Everyone knows these methods are not able, by themselves, to control favus of the scalp, hence the antibiotic deserves all the credit for cure of the disease.

No sign of intolerance to the antibiotic has been observed, in subjects with either favus or other types of tinea capitis. In one case we saw a transient erythema which spontaneously disappeared without stopping the drug. Systematic tests of kidney and haemopoietic function were performed before treatment was begun, and weekly as long as it lasted. Examination for albuminuria, Addis test and blood urea were carried out in all patients, but no abnormality was detected. In a few cases peripheral blood cell studies showed a slight reduction of erythrocytes. In half the cases the white blood cell count showed slight and relative deviation, either a polymorphonucleosis or a lymphocytosis. Up to the present, when using sufficient and effective doses, griseofulvin seems to be perfectly well tolerated.

In favus ringworm, checking the efficiency of treatment is easier than in any other type of ringworm infection, because plucking infected hairs and measuring their healthy part is possible at any time. Before therapy is begun the Adamson ring progresses into the hair bulb up to the non-keratinized part. From the 4th day of treatment the hair bulb is free of mycelial hyphae, and the Adamson ring has visibly drawn back, which proves that the inhibitory action of griseofulvin starts very early, nearly as soon as the first doses are absorbed. Then the basal part of the hair will quickly lengthen. The average length (from various hairs of one patient, and from hairs of various patients) will be 2.8 mm. on the 8th day, and 6.4 mm. on the 17th day; that is 0.4 mm. a day lengthening. This figure is exactly the same as the growth rate of healthy adult hair; this not only means a lessening, but an absolute arrest of the development of favus mycelial elements within the hair. Besides, the microscopical appearances could only allow one to suppose that this was correct, since a very sharp line of demarcation exists between healthy proximal and infected distal portion of the hair; and mycelial hyphae extending themselves within the healthy part, though progressively diminishing, can never be seen.

On the twenty-first day, when the course of therapy is over, the healthy emergent portion of hair is always long enough to enable shaving the infested extremities. It is well known that griseofulvin is fungistatic, but not at all fungicidal. Favus emphasises this fact conspicuously; at any time throughout the treatment, normal cultures of *Trichophyton Schönleini*, developing within the usual time, can be grown on agar slopes from the innermost part of the infested portion of the hair.

It is worth stressing the contrast between the absolute cures in the treatment of favus and the occurrence of relapses in the treatment of other tineas of the scalp, chiefly of microsporon species, in spite of the fact that the sensitivity of various Trichophyta and Microspora towards the antibiotic is almost the same. The greater power of the dissemination of spores of these last species seems to explain the differences, the more because recurrences appear in most cases not as a recrudescence of ringworm patches but as a reinfection after healing. Favus, being less contagious and producing fewer disseminating spores, does not carry the same risks. It is not impossible, however, that favus may be particularly sensitive to griseofulvin, as suggested by the dramatic healing of some instances of generalized favus developing in predisposed individuals when treated with the antibiotic. One of our clinical cases is worth recording; it is the story of a poor wretched girl suffering since childhood from a skin and mucous membrane infection by *Candida albicans*, which later became complicated by favus of the glabrous skin; the favus spread extensively despite all topical antifungal agents. In consideration of the abnormal resistance of the disease this patient was given exceptionally large doses of griseofulvin (1.5 gm. daily, then 2 gm. daily for two consecutive months). With this treatment favus mycelium which was shed in the keratin was completely eliminated, and all the favus lesions healed; but the monilial lesions did, naturally, not improve and were left unchanged. This patient is comparable to another one which has been recently reported by Benard, Drouhet, Nadal and Rene-Corail; it is the story of a man who had a favus lymphadenopathy. This patient was predisposed also; the infection which had been previously treated unsuccessfully by all known therapeutics, completely resolved when treated for a long period with griseofulvin.

Experience of such cases is still very limited, but they are worth reporting. The difference between the difficulty in treating widespread *Trichophyton rubrum* epidermophytoses and the rapid response in *Trichophyton Schönleini* infections leads us to think that *Trichophyton Schönleini* is more sensitive to griseofulvin than other dermatophytes.

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THE EFFECTS OF GRISEOFULVIN IN FAVUS

John H. S. Pettit

In a previous paper (1) the initial work that was performed in this trial has been described but it is considered necessary to include those findings, as well as more recent ones, in the present paper, as the earlier cases in the series have now been followed for a much longer period and a few cases need further comment. Beginning in February 1959, 230 patients were started on treatment with griseofulvin, of which 202 had favus and a further 28 had a form of "black-dot" ringworm. Several hundred other cases of favus were seen during the period under review and the reason for exclusion from the trial was invariably because the patients could not undertake to attend regularly for a weekly follow-up, either because of the distance they lived from the hospital or because they could not afford to stay away from work.

Unfortunately there is a tendency for the patients to stop attending if they are not satisfied with the speed of improvement, and it was also increasingly difficult to persuade them to attend for follow-up when no active treatment was being given. This made it necessary to attempt to trace a large percentage of the patients who had defaulted; in a city where most of the houses are in high-walled, unnamed alleys, and where none of the houses are numbered, this search was attended by tremendous difficulties; many patients disappeared into the warren of anonymous lodging and were never seen again. Because of these difficulties this paper contains a report on no more than 107 cases of favus and 19 of "black-dot" ringworm of the scalp.

Throughout the trial the diagnosis was always confirmed by the presence of Wood's light fluorescence in the hairs and the visualisation of the fungus under the microscope. The first cases were always cultured, but this practice was later abandoned as it was considered to be unnecessary. At the beginning of the trial all patients over the age of seven were given one gramme of griseofulvin daily for four weeks, and they were watched at weekly intervals; younger patients were given half of this dosage. At the end of the third week of treatment the appearance of a non-fluorescent root to the hair showed that the disease was being affected by the treatment. The first few patients were treated for five weeks but it soon became obvious that no more than four weeks' treatment was needed if the following scalp toilet was also used. At the end of four weeks the patients were instructed to shave the scalp and to wash it twice a day with warm water and soap. An attempt was made to follow every patient for a minimum of three months after the end of treatment and only fourteen of the cases reported were seen for less time. None is included unless it was followed for at least six weeks.

Quite early in the trial (Case 12) a boy of seven defaulted after two weeks' treatment with one gramme a day. He was found five weeks later and the fluorescence was definitely growing out. He was shaved and washed regularly and three months after his cessation of treatment there was no sign of infection. It soon became obvious that too many tablets were being given to the younger children. The dosage was therefore progressively diminished in an attempt to discover the minimum dosage; ultimately we went too far, and a boy of 12 (Case 112) who received half a gramme a day for a week, and others on similarly low treatment (Cases 160, 161, 166 and 167) all showed no benefit from the course. In every case a further and more adequate course produced satisfactory results, although the follow-up was not long enough to justify their further inclusion in this report.

In the light of the experience gained by this changing of dosage it was finally decided that the following dosage system is adequate for the average case of favus: —

AGE	DOSE
Over 15 years	250 mgms. three times daily.
7—15 years	250 mgms. twice daily.
Under 7 years	250 mgms. daily.

All patients receiving the drug for four weeks and shaving at the end of that time.

The table of results shows that of the 107 cases 75 showed no signs of relapse in the period of follow-up and can be considered as adequately treated. An analysis of the other thirty-two cases shows that most of them fall into one of two groups. The first of these unsuccessful groups include those patients who required further treatment but who were found, at that time, to have other infected members of their families or among their close friends. Patients No. 90 and 124 illustrate this problem well. Case 90, a 15 year old boy called Askar, was first seen on 23rd May 1959 and defaulted after three weeks' treatment with 500 mgms. a day. A month later he was seen again, the fluorescence was growing out, he shaved and was doing well when, on the 10th of July, he brought to the hospital his 10 year old brother Ali (Case 124) who had generalised fluorescence and scutula all over the scalp. Ali was treated with two tablets a day for four weeks, but two weeks after Ali had finished his course, on the 21st of August, Askar again showed a number of fluorescent hairs. These were manually epilated, but a week later there were many more and Askar was started on a further course on the 28th of August. Both patients then defaulted and were found again on 13th November when Askar still had an infection. Two weeks later Ali developed new signs of the disease. This family was instructive as it shows clinically that griseofulvin is simply fungistatic, and that the fungus in the hair of a patient under treatment is still infective.

Histories of this type were obtained in twelve of the thirty-two unsatisfactory cases. In eighteen of the other cases the need for further treatment could be ascribed to inadequate or unsatisfactory treatment. In some cases the patient defaulted before the end of treatment, and in others the head was never shaved; for example, Case 102, a girl of three years whose head was not shaved, and when seen two months later had widespread fluorescence at the roots in spite of previous adequate dosage.

The problem of inadequate dosage was particularly interesting in Cases 48 and 49, a girl of six called Khanum and her four year old brother Yussuf. Khanum had a severe favus of two years' duration, and her brother a milder infection which had been present for six months. They were both given 125 mgms. four times a day and failed to attend after two weeks' treatment. On follow-up three and a half months later, we found that the mother had not shaved either head, but that the older child, with the more severe original disease, was cured, while the boy still had about a dozen fluorescent hairs. As the dosage given was less than planned, and there was no shaving, the boy has been included in the "Inadequate Dosage" group, mainly because of our inability to detect another source of infection.

In the later stages of the trial doses were used which were, in retrospect, manifestly too small; for example, the family of Fatma, Beryukh Agha, Jaffa and Gholam Ali (Cases 146, 147, 148, and 149). Five weeks after the end of the course Fatma, who was 13, had not shaved and had relapsed. Six weeks after this the three others were all in need of treatment. Again it is possible that the dosage was adequate and the relapse in Fatma's case was due to incompetent scalp toilet and that the others were re-infected from her, but it was decided to include all these children in the "inadequate" group to be on the safe side.

TABLE OF RESULTS

Case Number	Age	Daily Dosage 250 mgm tablets	Weeks Treat-ment	Weeks Follow up	Result and Comment
1	7½	4	5	19	Cured.
7	10	4	5	20	Cured.
9	13	4	4	20	Cured.
10	2½	2	4	18	Cured.
11	6	4 2	4 1	17	Cured.
12	7	4	2	13	Cured.
15	5	4	5	18	Cured.
18	20	4	5	17	Cured.
19	12	4	4	17	Cured.
20	5	4	4	32	More treatment needed after 6/12, 2 affected sibs.
21	10	4	2	30	Cured.
23	10	3	4	15	Cured.
24	12	2	4	15	Cured.
25	5	2	4	15	Cured.
27	5	2	4	15	Cured.
29	12	3	3	16	Cured.
31	5	2	4	6	Cured.
32	7	3	4	16	Cured.
33	12	4	4	15	Cured.
34	4	2	4	7	Cured.
41	12	4	4	15	Cured.
42	3	2	4	13	Cured.
44	13	4	4	35	Reinfected, friend No. 139.
45	5	2	4	6	Cured.
47	9	3	4	24	Reinfected, sister No. 78.
48	6	2	2	16	Cured.
49	4	2	2	16	Not cured, ? inadequate.
50	7	2	4	15	Cured.
52	7	2	4	7	Cured.
53	3	2	2	20	Cured.
54	3	2	2	13	Cured.
56	12	3	3	14	Cured.
57	7	2	2	7	Cured.
58	12	4	2	12	Not cured, inadequate.
59	4	2	2	13	Cured.
60	2	2	2	13	Not cured, many sibs affected.
62	11	4	4	22	Not cured, sister No. 236.
65	25	4	4	15	Cured.
66	1¼	2	3	16	Cured.
67	6	2	4	12	Cured.
68	7	2	4	15	Cured.
70	7	2	4	6	Cured.
71	6	2	4	17	Cured.
72	7	2	4	7	Cured.
73	2	2	4	11	Cured.
74	13	2	4	17	Cured.
75	5	2	4	30	Not cured, ? reason.
76	9	2	4	23	Cured.
78	7	2	4	17	Cured.
81	6	2	4	14	Cured.
83	19	3	4	26	Scalp cured, nails improved.
86	5	2	4	27	Not cured, 2 sibs affected.
88	16	4	4	13	Cured.
90	15	2	3	28	Not cured, brother No. 124.

TABLE OF RESULTS (Continued)

Case Number	Age	Daily Dosage 250 mgm tablets	Weeks Treat-ment	Weeks Follow up	Result and Comment
91	9	2	4	22	Cured.
93a	16	3	1	Relapsed in four weeks.	
93b		2	4	13	Cured.
94	8	2	4	14	Cured.
98	7	2	4	21	Further treatment, sister No. 203.
99	5	2	4	20	Cured.
100	12	2	4	20	Not cured, sibs, 99, 125, 126.
101	5	2	4	23	Cured.
102	3	2	4	21	Not cured, never shaved.
104	2	2	4	13	Cured.
106	8	2	1	10	Not cured, inadequate.
107	7	2	4	13	Cured.
108	20	3	4	14	Cured.
110	4	2	4	22	Not cured, brother No. 134.
111	4	2	4	16	Cured.
112	12	2	1	Relapsed within six weeks.	
114	25	3 / 2	4 / 1	20	Not cured, never shaved.
115	13	2	4	23	Not cured, ? reason.
116	6	1	4	15	Cured.
117	12	1½	4	20	Not cured, ? inadequate.
118	9	1½	4	22	Cured.
119	3	1½	4	22	Cured.
122	16	3	4	6	Cured.
123	13	3	4	12	Cured.
124	10	2	4	19	Not cured, brother No. 90.
125	9	2	4	15	Cured.
127	9	2	4	7	Cured.
128	7	2	4	8	Cured.
129	19	3	4	12	Cured.
130	5	1	4	6	Cured.
134	2½	1	3	17	Not cured, ? inadequate.
135	6	1	3	16	Not cured, inadequate.
136	2	2	3	8	Cured.
137	7	4 / 2	1 / 2	10	Cured.
138	4	1½	4	10	Cured.
139	14	3	4	15	Not cured, friend of No. 44.
140	12	2	4	16	Cured.
146	13	1½	3	15	Not cured, inadequate.
147	9	1	3	15	Not cured, inadequate.
148	4	½	4	15	Not cured, inadequate.
149	8	½	4	15	Not cured, inadequate.
150	9	1½	4	13	Cured.
153	5	1	4	13	Cured.
155	3	1	4	13	Cured.
156	7	1	4	13	Cured.
159	7	1½	4	10	Cured.
160	6	2	1	6	Not cured, inadequate.
161	4	1	2	6	Not cured, inadequate.
164	7	1	4	10	Cured.
165	16	4 / 2	1 / 2	8	Cured.
166	13	3	1	10	Not cured, inadequate.
167	6	1	2	10	Not cured, inadequate.
174	5	1½	1	10	Cured.

There remain only two other patients for discussion, Cases 75 and 115. No. 75, a girl called Maliheh, was five years old, and had four patches of red scaliness on the scalp with a considerable number of fluorescent hairs in each patch. She was given two tablets a day for four weeks, and shaved at the fourth week. Her 13 year old sister (Case 74) had similar treatment at the same time and recovered with no sign of re-infection over a period of four months. Maliheh, however, relapsed after sixteen weeks; examination of the whole of the family showed no other contact; she did not go to school and rarely left the compound.

Case 115, Rasool, was thirteen and had suffered from the disease for 7-8 years. He had a patch of cicatricial alopecia about five inches in diameter on the crown of the head, and the majority of the remaining hairs were infected. He was given two tablets a day for four weeks and attended with the utmost regularity. Nine weeks after the end of treatment the scalp became a little scaly and in spite of regular washing fluorescence appeared at the end of a further four weeks. Here again there was no proof of contact with infection. In both of these cases the patient received what was considered to be adequate dosage, and although the period before the re-appearance of the disease was 3-4 months, these two have been placed in a group of their own as relapsed cases.

The nineteen cases of "black dot" ringworm are included in this paper with some diffidence. The facilities were not available for me to identify the causative fungus. It is presumed that the patients were suffering from trichophyton sulphureum or trichophyton violaceum infection. In all cases the fragility of the hair which causes it to break at scalp level, seemed to disappear within a very few days of the starting of treatment. The usual routine was followed and the scalp was shaved at the end of four weeks. From this time onwards it was always impossible to recognise the site of infection as the hair grew normally. The only test of cure other than this was a "shot-in-the-dark" sampling of hairs. The inability to recognise the causative fungus and also the impossibility of demonstrating its disappearance in these patients produce a completely inadequate report, but I feel it is of importance to put on record the clinical impression that griseofulvin is of value in these cases.

Summary

107 cases of tavus were treated with griseofulvin. 75 of these were cured, 12 were re-infected by members of their family or by friends, 18 received an inadequate dosage or failed to carry out the instructions, and only two can be considered as relapsed cases. Nineteen cases of "black-dot" ringworm were also cured. The recommended dosage in this series is 250 mgms. three times daily for four weeks for patients over sixteen, 250 mgms. twice daily between the ages of seven and sixteen, and a daily tablet for those under seven years.

Acknowledgements

The great generosity of Glaxo Laboratories Ltd. in supplying all the griseofulvin must be recorded. The work was performed while the author held the chair of dermatology at the University of Tabriz, Iran.

REFERENCE

(1) Pettit, J. S. S. (1960) *Brit. J. Derm.* **72**, 179.

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GRISEOFULVIN IN TINEA CAPITIS

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Since March 1959 griseofulvin has been given to 91 children with tinea capitis (*Trichophyton violaceum* in 30, *Microsporum canis* in 28, *Trichophyton tonsurans* in 15, *Trichophyton schoenleinii* in 8, *Trichophyton megninii* in 1, *Trichophyton mentagrophytes* in 1, *T. violaceum* associated with *M. canis* in 2 and unidentified species in 6). Of the total, 65 children were treated in the hospital and 26 as out-patients. Several daily dosage schedules were tried without relation to age or weight. In 72 patients there was administered a constant daily dose: 1 gm. in 25; 0.75 gm. in 9; 0.5 gm. in 34 and 0.25 gm. in 4. In the remaining 19 children the daily dose was changed during treatment: in 3 cases, after a period of 14 to 18 days on 0.75 gm., the dose was reduced to 0.5 gm. in 2, and to 0.25 gm. in 1. In 16 children treated with 0.25 gm. during 6 to 46 days, the dose was raised to 0.75 gm. in 2, and to 0.5 gm. in 14, when it was verified that some cases did not heal within the usual period found for higher doses.

Concomitant local treatment was not generally used, except when intensive scaling, crusts or suppuration were present. In fact, it had been previously noted that local treatment did not shorten the duration of treatment with griseofulvin, and made the microscopic examinations more difficult. Shaving of the scalp followed by washing was repeatedly performed during the treatment in the majority of cases, and it made the mycological examinations easier and contributed to the elimination of parasitised hairs, which was particularly found in cases of favus. Clinical and mycological control was performed weekly in all cases during and after the treatment.

The following results were obtained:

(a) Cure confirmed one to eight months after treatment in 71 cases;

(b) Reappearance of the infection one to ten weeks after cessation in 19 cases;

(c) Persistence of the infection after eighty-four days of treatment in 1 case.

Dosage and Duration of Treatment

Table I summarizes the results obtained in 72 children treated with a constant daily dose, and Table II gives the findings in 19 children with variable doses during treatment.

Although the numbers are not equivalent in the groups treated with several dosages, the average duration of treatment and the period of sterilization of the scalp were

Dose in gms.	Number of cases	Age of patients (years)	Average duration of treatment (days)	Average period to sterilization (days)	Results	
					Cured	Recurrence
1.0	25	3–13	33.0	26.4	21	4
0.75	9	3–12	35.8	28.8	9	–
0.5	34	1–13	33.0*	28.5*	26	8
0.25	4	2–5	39.0	37.0	4	–

*Except one case of kerion by *T. mentagrophytes* which cured after 14 days.

TABLE 1.—RESULTS WITH CONSTANT DOSAGE

* Supported by a grant of 'Institute de Alta Cultura', Ministry of Education, Lisbon.

Dose in gms.	Number of cases	Age of patients (years)	Average duration of treatment (days)	Average period to sterilization (days)	Results		
					Cured	Recurrence	Failure
0.75 + 0.5 or 0.25	3	4–7	33.7	31.3	3	–	–
0.25 + 0.75 or 0.5	16	2–6	45*	43.3*	8	7	1

*Except 1 patient four years old with lesions by M. canis, which remained positive after 84 days of treatment (12 with 0.25 and 72 with 0.5 gm.).

TABLE II. — RESULTS WITH VARIABLE DOSAGE DURING TREATMENT

similar in patients treated with 1 gm., 0.75 gm., and 0.5 gm. daily. Those figures were a little higher when 0.25 gm. per day was administered. In the cases treated initially with 0.25 gm. daily followed by 0.75 or 0.5 gm. it was necessary to prolong treatment. However, considerable variability in the treatment's duration and of the sterilization's period was recorded (between 14 and 63 days, and between 16 and 61 days respectively).

Analysis of Failures

Reappearance of the infection after treatment was observed in 19 children out of 91 treated (20.9%), whilst one patient remained infected for a rather prolonged period. It is always difficult to establish whether reappearance of the infection after the treatment indicates relapse or reinfection. However, reinfection can be suspected when there is either: (i) an aetiological agent different to that previously identified; (ii) localization different to that registered before; (iii) a prolonged period with negative mycological examination between the cessation of therapy and the reappearance of lesions; or (iv) the existence of a source of infection which could reinfect the patient.

The analysis of the above related 19 patients revealed:

(i) a different aetiological agent in 1 case. (*T. schoenleinii* in the first lesions and *M. canis* later);

(ii) different localization in 12 cases;

(iii) the period between the cessation of therapy and the reappearance of lesions varied from one to ten weeks: one week (1 case); two weeks (1 case); eight weeks (4 cases); ten weeks (2 cases).

(iv) probable sources of reinfection in 10 cases: brothers with tinea capitis in 6; mother with onychomycosis in 1; utilization of cap used before the treatment in 3.

Based on these arguments 12 cases were considered as reinfections. There was no evidence to classify the remaining 7 cases as reinfections but we could not be certain that they were relapses. In the patient who remained positive after 12 days with 0.25 gm. followed by 72 days with 0.5 gm. it was admittedly insufficient dosage. The reappearance of infection occurred with similar frequency in patients treated in the hospital (25.9%) and in out-patients (23.1%) but in the first group the new lesions appeared after leaving the hospital. Thus they had had an opportunity for being reinfected.

The reappearance of infection did not seem related to age, but occurred generally in younger children (from 2-8 years) and never after the age of 8. There was not found any relationship with the aetiological agent, which was *M. canis* in 8; *T. violaceum* in

6; *T. tonsurans* in 2; *T. violaceum* associated with *M. canis* in 1; and unidentified species in 1. It is remarkable that in 8 cases of favus (*T. schoenleinii*) reappearance of lesions did not occur except in 1 child reinfected by *M. canis* five weeks after the treatment's interruption.

One of the 20 cases of failure was not traced but the other 19 were treated: 8 cases by X-ray epilation; 11 with second treatment of griseofulvin. 7 out of those last are still under observation, 2 cured and reappearance was again registered in 2.

Criteria of Cure

After several weekly negative mycological examinations (microscopic and culture) the persistence of clinical alterations (scaling, alopecia or crusts) was observed in the majority of cases. For that reason the criterion of cure adopted was based on mycological findings. Excluding the case which remained positive after 84 days, treatment was interrupted:

(a) After the first negative examination in 38 cases.

(b) After the second negative examination in 46 cases.

(c) When there were seen only few hyphae in the extremity of some hairs in 6 cases, in which the scalp was shaved at that moment.

The results obtained are shown below:

Interruption	*Number of Cases*	*Cured*	*Recurrence*
After the 1st neg. examination	38	27	11
After the 2nd neg. examination	46	39	7
With fungi on the tips	6	5	1
TOTAL	90	71	19

Hence it can be seen that the reappearance of infection did not seem to be related to the date of interruption of treatment. However, as the demonstration of parasites is sometimes difficult during the last days of treatment, it is more prudent to continue the tablets until two weekly successive negative examinations. It is remarkable that four cases of favus healed after interruption of treatment when fungi were still present in the extremities of some hairs, which were removed at the time they were found. Since, in some instances, the reappearance of lesions was noted ten weeks after cessation, we recommend periodic surveillance of the treated patients for at least three months.

As regards the tolerance of griseofulvin, we did not observe any clinical manifestation which could be attributed to a toxic effect of griseofulvin. Blood and urine examinations before, during, and after the treatment did not reveal any modifications in 65 children.

Comment

The results of this experience with griseofulvin are similar to those reported by others, except that the number of failures was higher. Thus, analysis of failures seems very important. Reappearance of infection after treatment with any etiotropic drug may be due to reinfection or to relapse, which is frequently difficult to distinguish. Reinfection was admitted in the majority of failures of this trial. As *tinea capitis* is endemic in Portugal, children have an opportunity to be reinfected after treatment.

Because griseofulvin represents a great simplification of the therapy of *tinea capitis*, there is a natural tendency to neglect prophylactic measures during and after treatment.

This could explain the frequency of reappearance of lesions in this experiment. For that reason the necessity of active sanitary education and prophylactic measures, including the investigation of all the possible sources of infection, should be emphasised.

As regards the tolerance of griseofulvin, we did not observe any clinical manifestation which could be attributed to the toxic effect of the drug. Blood and urine examinations before, during and after the treatment did not reveal any modifications in 65 children.

Local Treatment of *tinea capitis* with griseofulvin

Nineteen children with *tinea capitis* and one adult with chronic *tinea corporis* were exclusively treated for four weeks by daily application of a paste containing griseofulvin at 1% (5 children), 3% (6 children and 1 adult), and 5% (8 children). Lesions were washed daily before each treatment.

After four weeks, no improvement was noted in the lesions of 18 children and in the adult where fungi were easily demonstrated. In one child with *tinea capitis* (*T. violaceum*) the mycological examination was negative after the second week of local treatment with griseofulvin at 5%; medication was then interrupted and the patient was kept under weekly observation. Mycological examination remained negative for four weeks but was again positive in the fifth week.

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DISCUSSION

PROF. VANBREUSEGHEM: There have been so many interesting papers presented this afternoon that I will limit myself to one single remark about this vexed question of the treatment of favus by griseofulvin. I think this question can be clarified, if not understood, if we remember the rule written on the wall of a community. This rule said: "In this house we are on a footing of equality, but some people are more equal than others."

Actually, I intend to try to produce evidence that when you feed griseofulvin to some animals the griseofulvin is not equally distributed in the nails and, after three weeks' treatment, not more than fifty per cent of the nails are protected against invasion by dermatophytes. What happens later on I do not know, but I think it is an interesting aspect of this question.

DR. PETERKIN: I want to make a very short remark on side-effects about which Dr. Calnan has spoken in his very interesting paper. I found that if I did not say to the patient that the drug would produce an effect, no side-effects were observed. I noticed that anyone who said: "I have a very weak stomach" inevitably had diarrhoea or indigestion, even hiccoughs. The only person who could not go on with the treatment was a lady who said: "I'm allergic to any drugs; I'm always sick"; and she was. In two cases I thought the results were especially interesting. After a year's treatment in each case a man developed a black, hairy tongue and a woman developed glossodynia and angular stomatitis. I wonder if that has been found by others in griseofulvin treatment?

Turning to Dr. Pettit's fascinating paper, and considering his relapsed cases, we used to have a fair number of cases of favus in Edinburgh, and we found that most cases came from Leith and the port areas. This was because favus was brought in from the Baltic areas by rats and mice. I wonder if Dr. Petit has explored the possibility of the relapse being due to rats, mice, cats, dogs, or even that pleasant animal the desert rat, or jerboa, which carry this infection?

DR. PETTIT: If I may answer Dr. Peterkin, the jerboa is not present in Azerbaijan; but it is present in the south of Iran. The dog is banned by Mohammed and is never allowed in the house; the only contact children have with dogs is to throw stones at them. Cats are not kept as pets. There is quite a considerable number of rats about, but that is the only animal that is a possible vector. Unfortunately I was rather swamped by patients and was not able to go into that aspect.

DR. MARRE: I would like to ask Dr. Pettit whether the relapsed areas were the same as the areas of the original infection?

DR. PETTIT: Almost invariably one found the same site affected in the ones I have put under the heading of inadequate dosage. If they relapsed through re-infection from other members of the family it often appeared in other parts of the scalp.

SURG. CMDR. SCUTT: I would like to ask a question about whether any clinical trial has been carried out to show exactly what is the minimal dosage level of griseofulvin? I am not talking about favus cases so much as about long-term cases of infection of the nail. It is obvious that with a very expensive drug such as this, six months' treatment for infection of the nails costs something like £21. It has been suggested that you could cut that down quite considerably in two ways, one by surgery of the nails and the other by cutting the dose rates, by giving 2 gm. twice a week, or even possibly 1 gm. twice a week. As far as I can see we do not really know whether 1 gm. a day is what is absolutely necessary for six months; or whether 1 gm. is necessary for one month, followed by a reduction. I think this is particularly important also in view of the fact that general practitioners use this drug very widely, particularly for quite commonplace

fungal infections of the feet such as interdigital toe lesions which we know now do not actually respond to griseofulvin at all. It does seem to me that it would be right that they should understand very definitely that this drug is not suitable for the treatment of that sort of case.

DR. THORNE: I wonder whether people have paid enough attention, in *Trichophyton rubrum* infections, to following-up family contacts? I have not heard much about it from others. When I take a case I try to see other members of the family where I can; and where it is not possible I get them to bring clippings from the nails of other members of the family who might have nail infections. There have been one or two instances where we have found extra cases by that means. Further, if this infection is going on in the household, how do we expect to get patients free of it? I do not know whether there is any way in which we can co-operate with the public health authorities who, after all, in the past have done some good work with respect to head and body lice.

Another point is that I find many hospitals give inadequate pathological facilities for finding fungi of one sort or another. I think if we could have some area pathologists who would take special interest in this subject it would be helpful.

I should like to know if anyone can tell us how long *T. rubrum* can live in a household, on mats and carpets and so on, possibly causing infection? I think that is another point we want to discover, if it is not already known.

As far as the low blood count is concerned, is it not possible that many of our patients suffering from any disease have low polymorph counts? I think far too often we start doing blood investigations with new drugs and then get unduly worried because the count is low. We should do these investigations sometimes on ordinary people and we might find some rather bizarre results which might take some explaining, but the patient will be none the worse for them.

DR. JANKE: I would like to make a remark on the question of side-effects. We have observed in several cases the provocation of *Candida.* Because of exacerbation and provocation of *Candida mycosis* which we observed under penicillin therapy, we suspected that perhaps griseofulvin might have a similar stimulating effect on *Candida.* Experimentally, such stimulation could not be observed. As test systems we used the culture diameter of growing colonies of *Candida albicans* on a medium containing increasing amounts of griseofulvin. Readings were taken after one, two and three days in comparison with control cultures on griseofulvin-free media. It was observed that in all the concentrations tested, only a minimal, clinically-insignificant growth inhibition of *Candida albicans* occurred, and no stimulation. I would like to ask Dr. Calnan whether he has found any evidence of stimulation of *Candida mycosis*?

DR. CALNAN: No.

THE CHAIRMAN: We saw certain mycotic infections which looked as though they were produced by *Trichophyta* but they were *Candida* cases. Repeated cultures always showed *Candida.*

THE EFFECTS OF GRISEOFULVIN ON TINEA UNGUIUM

G. A. Grant Peterkin

Since 1957 I have been interested in the treatment of ringworm in man with griseofulvin, as in that year I first heard of the report by A. R. Martin (1956) who, under the supervision of Dr. M. H. C. Williams, had treated by means of topical griseofulvin two laboratory workers who had become infected with T. mentagrophytes in the course of experiments with guinea-pigs given the drug orally. In a controlled series of cases of *tinea pedis*, occurring in a boarding-school, Brewster and I (1958) found that 1% griseofulvin ointment was no more effective than two popular fungicidal ointments, though I mentioned that "it seems possible that in the future, fungus infections of the skin may be eradicated by some antibiotic given parentally."

However, the report by Paget and Walpole (1958) of colchicine-like effects on bone-marrow and seminal epithelium following huge doses given intravenously or intra-peritoneally appeared to warn against the use of griseofulvin by mouth, and it was

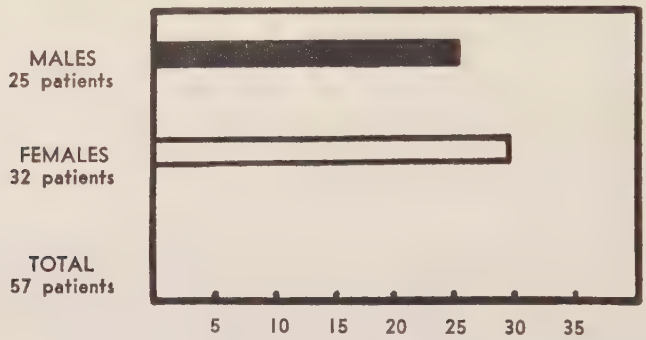

Fig. 1. Number and Sex Incidence.

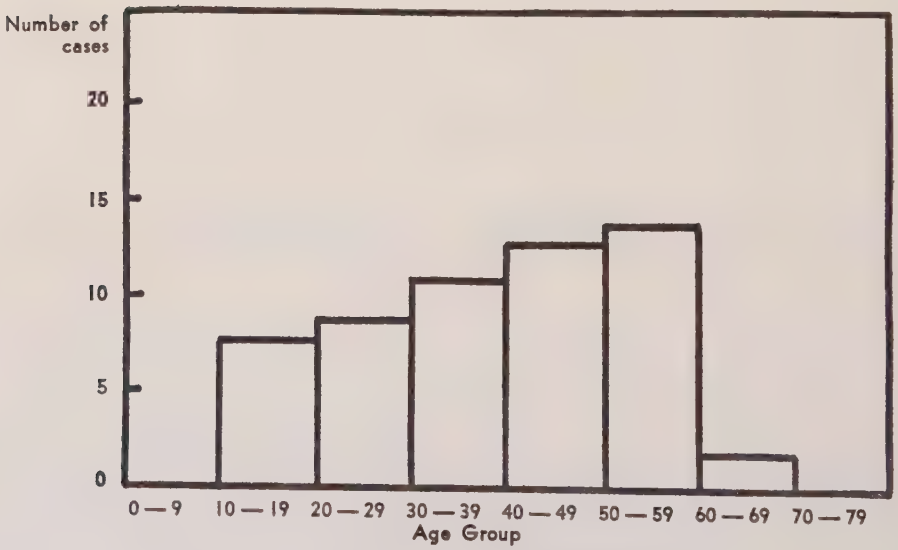

Fig. 2. Age incidence of tinea unguium cases.

not until December 1958 that the present series of patients had commenced treatment. By this time the work of Gentles (1958), Williams, Marten and Sarkany (1958) and Blank and Roth (1959) had proved the low toxicity of the drug.

This is a resumé of the results of treatment in fifty-seven cases treated during the twelve months from January to December 1959, their progress being reviewed up to the month of March of this year. The dosage employed was 250 mgs. four times a day.

Number of Patients, Sex and Age Incidence

Fig. 1 shows the number of cases treated and the ratio between males and females. Fig. 2 shows a tabulation of the ages, the youngest patient being eleven and the oldest sixty-five.

Social Aspects

Fourteen patients in this series had close relatives who had active fungus infections of the feet, confirmed by personal inspection, and doubtless a much higher percentage would have been uncovered had it been possible to examine near relations of all the patients, but this was impossible, for various reasons. Of the fifty-seven patients, sixteen (i.e. about 28%) were seen by me privately—which seems to be a high incidence. Of the forty-one patients treated at hospital, only twelve could be classified as unskilled workers, and of these three were coal-miners and three had seen service as soldiers in India.

Diseases mistaken for and treated as *Tinea Unguium*

One of the most interesting facets of griseofulvin therapy was the vast amount of this drug prescribed for diseases other than ringworm, with a resultant great waste of public money. Many patients referred to the dermatologist for treatment of diseases affecting the nails had taken griseofulvin for a month or more before an accurate diagnosis had been made. No doubt all dermatologists could quote similar cases. Examples encountered were:—

(1) Psoriasis of the nails, which often presents real diagnostic difficulties, and which may occur with onychomycosis. Of the present series, two also had psoriasis.

(2) Perionychia due to *Candida albicans.*

(3) Eczema with associated nail changes was only rarely diagnosed as *tinea.*

(4) Dermatitis Repens and Acrodermatitis Continua, which in one patient involved the toes as well as one finger.

(5) Onychogryphosis was, in two cases, thought to be a fungus infection.

(6) Dystrophia Unguium of various types was treated as a fungus infection. One man was invalided back from West Africa as a case of *Tinea Unguium.* Actually, he had a Congenital Ectodermal Defect, precipitated by mepacrine.

(7) Lupus Erythematosus and Lichen Planus were other disorders similarly mistaken for onychomycosis.

Cultural and Microscopic Findings

T. rubrum was the fungus most frequently grown, though *T. mentagrophytes* was not uncommon. Others found were *T. sulphureum, T. ferrugineum,* and *Scopulariopsis brevicaulis.* In each case the diagnosis was confirmed by microscopic examination, and the criterion of cure selected was the finding of three negative potassium hydroxide specimens taken at four to six week intervals, from nails which had at one time been heavily infected.

Fingernails	Up to 30th June	1st July to 31st Dec.	Total
Cured	22	5	27
Improved	0	10	10
Unchanged	1*	0	1
Stopped treatment	2	0	2
	25	15	40

Fig. 3. Results in 40 cases of tinea unguium.

* C. albicans only cultured; yeasts and mycelial threads microscopically.

Results of Treatment

Fig. 3 gives the figures for the cases with finger-nail involvement, in which the cure rate is so much higher than in the toe-nails. It seemed likely that cure of the finger-nails would eventually be obtained in all patients who persisted with the treatment.

Toenails	Up to 30th June	1st July to 31st Dec.	Total
Cured	9	0	9
Improved	15	24	39
Unchanged	1*	1†	2
Stopped treatment	4	0	4
	29	25	54

Fig. 4. Results in 54 cases of tinea unguium.

* C. albicans only cultured; yeasts and mycelial threads microscopically.
† Big toenail clinically unchanged; microscopic examination negative on two occasions; two negative cultures.

The results in the toe-nail cases (Fig. 4) are much more depressing. Although almost all showed considerable improvement, and some seemed to be cured, yet in many the rate of healthy nail growth had slowed up, and it appeared possible that even constant therapy for over twelve months would fail. In six cases the drug was stopped for six weeks after a band of healthy nail had appeared; in each case below this band the infected nail plate appeared again, and this may well happen with toe-nails when prolonged treatment has been stopped after apparent cure. Most patients are not enthusiastic about continuing drug-taking once their symptoms are relieved and their finger-nails are again presentable. Surgical removal of the toe-nails was resorted to in three cases, which have not been included in this series. In many of the women the toe-nails were so deformed for various reasons that the clinical appearance could not be relied on.

Number of patients in series	57
Number of patients having side-effects:—	15
1. Gastro-intestinal (e.g. thirst, nausea, indigestion or diarrhoea) ...	10
2. Headache	3
3. Urinary Symptoms	2
4. Temporary Urticaria	1
5. Oral Lesions (black hairy tongue; glossodynia & angular stomatitis)	2

Fig. 5. Toxic Effects

Toxic Effects

This afternoon, these will be dealt with by abler hands than mine. Fig. 5 shows details of the fifteen patients, of whom only one had to stop treatment entirely.

Summary

The results of oral griseofulvin, given in a dosage of 1 gm. daily, in a series of fifty-seven patients with ringworm of the nails are tabulated. Of these, many had close relatives infected with *tinea pedis*.

Hundreds of pounds have been wasted by the prescribing of this drug for patients who did not have *tinea* at all, but had other nail conditions mistaken for it.

Toxic side-effects were usually of a mild nature, when they did occur; only one patient could not continue to take the drug.

Acknowledgements

I am indebted to Drs. J. M. Mungavin and C. C. Downie of Imperial Chemicals Ltd. for advice and the generous supply of griseofulvin, and to Dr. A. R. Martin of Imperial Chemicals Ltd. for culturing nail specimens.

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TREATMENT OF NAIL INFECTIONS WITH GRISEOFULVIN COMBINED WITH ABRASION

H. P. Jeremiasse

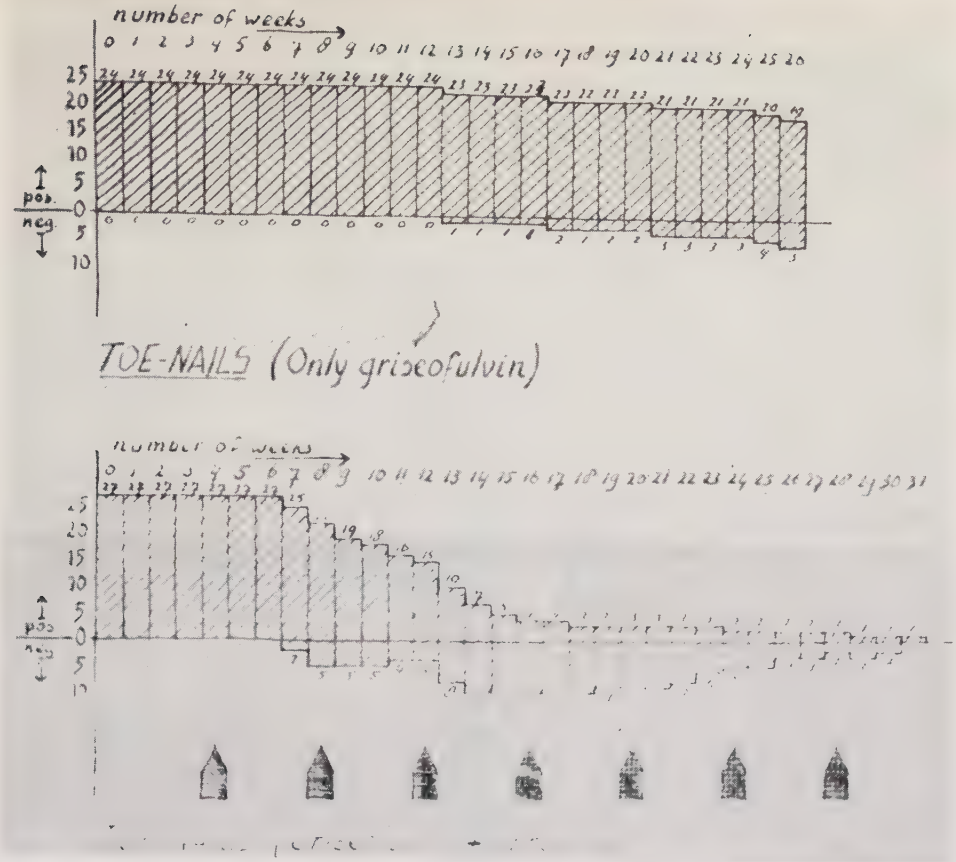
The purpose of this presentation is to report to you on a combined treatment for nail infections caused by *Trichophyton rubrum* with griseofulvin and abrasion of the nails. Before going further into this, I shall very briefly explain how we arrived at this form of treatment.

In our first group of patients with chronic *Trichophyton rubrum* infections of hands and feet, griseofulvin was given in a dose of 250 mg. four times a day without any form of local treatment. During the course of observation it became apparent that in general the skin of hands and feet cleared satisfactorily, although relapses and persistence of interdigital cleft infections were noted. One thing that struck us was the exceedingly slow improvement of toe-nails. This is illustrated by our results of 6 months of continuous griseofulvin treatment on 27 patients. After this half-year period only 5 out of 24 patients showed negative scrapings of the toe-nails, whereas 23 out of 24 patients had negative findings of the skin of the feet. Ten out of 11 patients had negative finger-nails, and 13 out of 15 patients had negative scrapings of the skin of the hands (the 2 positive cases here were relapses). Clinical improvement roughly paralleled the microscopical findings so that after this half-year period the skin and finger-nails of most patients had greatly improved or even cleared, whereas the toe-nails showed only very moderate improvement with positive microscopy or culture. We therefore felt that some form of supplementary treatment for toe-nail infections was required lest griseofulvin administration be continued beyond economical limits.

One might postulate that the griseofulvin incorporated in the keratin of the nails forms a barrier against fungal invasion so that, once this barrier has been formed, the fungi will be pushed out in front of the newly growing nail with its deposit of griseofulvin. Although this is a matter of great theoretical and practical interest, we felt, however, that there are so many unknown factors which determine the direction of the equilibrium between the invasive powers of the fungi and the resistance against this invasion by the griseofulvin-incorporated horn, that for the time being the patient's best chance of a speedy recovery lies in complete removal of the infecting organisms as soon as possible. For nail infections, this could be done by surgical extirpation of the affected nails. Although theoretically this seems the safest way to get rid of all infectious material, the method in our opinion has too many disadvantages to be of real practical value. For surgical removal of the nails the patient has to be hospitalized when suffering from a widespread infection. Furthermore, this form of treatment is very uncomfortable. We therefore used a simpler form of supplementary treatment which is more acceptable from the patient's view.

One month after starting oral griseofulvin treatment, as much as possible of the affected toe-nails was removed by simple abrasion with a dentist's drill. This procedure was repeated at monthly intervals until microscopical findings of nail scrapings became repeatedly negative. Although we are fully aware that the deeper parts of the nails cannot be reached as thoroughly with this simple method as with a surgical avulsion, we felt this was no real problem because the deep and hidden parts of the nails, which cannot be reached by abrasion, should be cleared by the blood-borne griseofulvin by the time we started abrasion.

Fig. 1 is a diagrammatic representation of the microscopical findings of 27 patients we have treated with this method. On the top you see the results of 24 patients who were treated with griseofulvin exclusively. At the bottom the results with the abrasion technique are plotted. This is a cumulative diagram because not all patients have been followed for as long as the time-axis goes. The black arrow marks beneath indicate the abrasions.



The first abrasion does not seem to make much difference. Perhaps we started abrasion too early in treatment. Between the 3rd and the 4th abrasion, however, there seems to be a rather sharp decrease in the total number of positive findings. At the time of the 3rd abrasion, 15 out of 19 patients were still positive, at the time of the 4th abrasion only 4 out of 14. When one compares the results of these two groups after 4 months, in a four-fold table we arrive at a X^2 of 162.

One can also see from this diagram that some patients seem to be very resistant to this form of treatment. One patient, for example, is still positive after 7 abrasions. Remarkably enough, her toe-nails are almost completely cured clinically. This brings us to the subject of clinical improvement with the abrasion technique. In general it can be said that nails seem to grow out better and sooner after removal of the mycotic material. This can be demonstrated on a few patients with equal involvement of the nails of both feet, where only one foot was treated with abrasion and the other foot was left without this treatment as a control. In most patients the improvement on the side of the abrasion was better than on the control side.

This is only a preliminary report. We realise that the time of observation and the number of patients is too limited to allow for a definite evaluation of this form of treatment, but feel that the results to date have been encouraging and worthwhile.

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TREATMENT OF ZOOPHILIC RINGWORM IN MAN (T. VERRUCOSUM AND T. MENTAGROPHYTES INFECTIONS)

C. H. Whittle, R. H. Champion and J. L. Moffatt

Ringworm of animal origin is common in rural areas (Ford, 1956, Whittle, 1956, Rook, 1956, Cochrane and Tullett, 1959). *T. verrucosum* infections are invariably derived from calves or their environment: *T. mentagrophytes* occasionally from calves (Ainsworth and Austwick, 1955) but mostly from other animals; the precise origin is often obscure, rats and mice probably acting as a reservoir (Georg, L., 1956; La Touche, C., 1960). The lesions are frankly inflammatory and have a natural tendency to heal spontaneously.

Three questions arise: (1) Will griseofulvin hasten the cure? (2) Will it reduce the severity of the infection? (3) Will it prevent the hypertrophic scarring which occasionally follows a kerion?

The effect of oral griseofulvin alone has been observed on 18 cases, 6 of which had not completed their treatment at the time of drafting this paper, but their response is known to confirm the figures given. They were country dwellers, and all except one woman and two children aged 11 and 13 worked on the land. Kerions were present in 10 of the patients. Only one patient had had ringworm before, but he had had several lesions during the previous nine years.

The series of 12 cases—8 of *T. verrucosum** and 4 of *T. mentagrophytes* infection—have been compared with a random series of 40 cases—26 of *T. verrucosum* and 14 of *T. mentagrophytes*—treated by the older topical applications. No precautions were taken against re-infection other than a dry dressing to prevent spread on clothing.

Results of Treatment

1. Subjective improvement, such as loss of itching, soreness and tension, may occur in 48 hours.

2. Objective improvement with diminution of swelling and redness is often seen within 3 days (Figs. 1 and 2).

3. The lesion may, however, extend for a few days despite full doses of griseofulvin. This is illustrated by Case 3, a child aged 11. Comparison of the two photographs (Figs. 3 and 4) shows considerable increase in size of the lesion in the week's interval in spite of 1 gramme of griseofulvin daily, but Fig. 4 shows also scaling, fading and resolution. Without daily observation we can only surmise what happened, but it is probable from observation of parallel cases that the lesion increased rapidly in size in the two or three days before the griseofulvin reached the layer of keratin which contains the growing fungus.

4. Healing may be complete in 14 days. It was so in 6 cases, 2 of which had kerions (see Figs. 5 and 6). Kerions naturally take longer to heal than superficial lesions; but even so there is considerable variation in response between patient and patient.

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* The fungus from kerion lesions may frequently fail to grow in culture. In three cases in which cultures were sterile the patients were at the time actually treating calves infected with ringworm.

Duration of Treatment

Treatment for longer than 14 days is advisable if relapse is to be avoided. Two patients clinically cured in 14 days relapsed 14 days later after the drug had been discontinued. The relapse was almost certainly due to re-infection from the clothing. In one, the relapse showed as three tiny widely separated papules arising on the sites of the three healed lesions (Figs. 7 and 8). Positive films and cultures confirmed the nature of these very early new lesions.

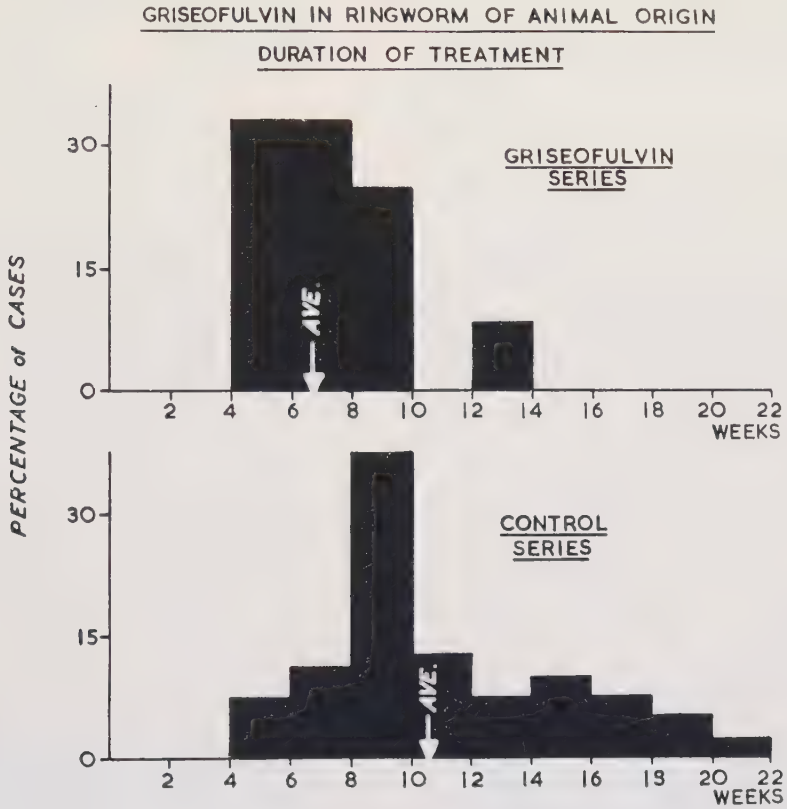

Chart 1. Showing overall and average time taken to heal in the Griseofulvin series compared with the control series.

Recovery Time

Chart 1 gives a comparison of rate of recovery of the griseofulvin-treated series and the control series. It shows:

1. The griseofulvin group is shifted to the left, indicating a more rapid recovery rate. The average is 6.3 weeks as compared with 10.6 weeks in the control. This is statistically significant ($0.005 > P > 0.001$).
2. The highest percentage of cases on griseofulvin healed in the 4-8 weeks period, the controls in the 8-10 weeks period.
3. The scatter in the control series is much greater than in the griseofulvin series, 4-22 weeks as compared with 4-14 weeks.

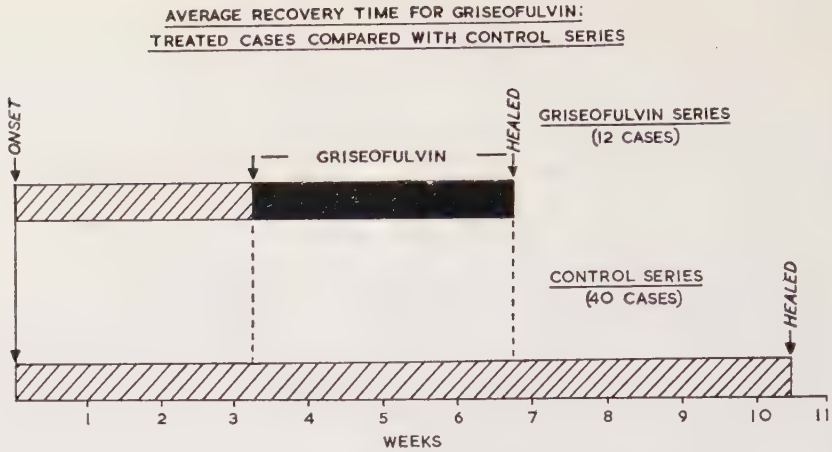

Chart 2. Showing halving of recovery time in Griseofulvin treated cases compared with the expected time of recovery in the control series.

Chart 2 illustrates more clearly the speed in recovery of the griseofulvin series. By plotting the average time of recovery of the griseofulvin series against the expected average time of recovery starting from the same point on the control series, the time for recovery on griseofulvin appears to be half that in the control series.

Morphological Changes in the Fungus

Distorted forms were seen in samples taken from at least two patients with lesions which were beginning to show response to the drug. Large club-shaped hyphae and gross increase in thickness and refractility of the hyphal wall were noted, features which have hitherto been attributed to the effects of the griseofulvin on the growing hyphae. However, somewhat similar forms have been seen in spontaneously healing lesions which have not been subjected to griseofulvin treatment, and it seems likely that such shapes may result from the presence of substances inhibiting fungal growth actually elaborated by the normal tissue reaction in the skin. This does not detract from the therapeutic value of griseofulvin, but it may afford a clue to a line of research, probably already in train, on the mode of action of the drug. It is accepted that griseofulvin rapidly builds up a barrier, but it may well be that this barrier is essentially not very different from the barrier built more slowly, but in the end as effectively, by the natural defences of the body against these two fungi.

Dosage

1-2 gm. daily in divided dose; for superficial lesions at least 4 weeks, and for deep pustular lesions up to 10 weeks. Treatment is continued 1-2 weeks after clinical cure, otherwise relapse is frequent.

Summary

1. The effect of oral griseofulvin alone on zoophilic ringworm in man has been observed in 18 cases. Six of these have been omitted from the statistics because they arrived too late to complete treatment; but it is now known that they confirm the findings of the other 12 cases. The series, 8 of *T. verrucosum* and 4 of *T. mentagrophytes* infection, has been compared with a series of 40 cases—26 of *T. verrucosum* and 14 of *T. mentagrophytes*, treated by the older topical applications.
2. Griseofulvin reduces the average time of recovery to half the time forecast for treatment by the older methods. The average total duration of infection of 6.3 weeks as against 10.3 weeks is statistically significant (0.005 P 0.001).

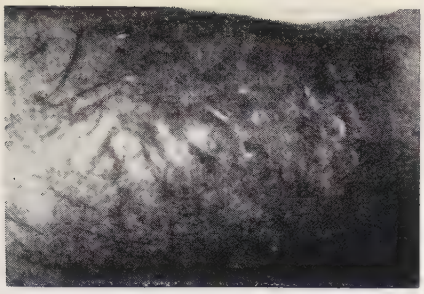

FIGURE 1

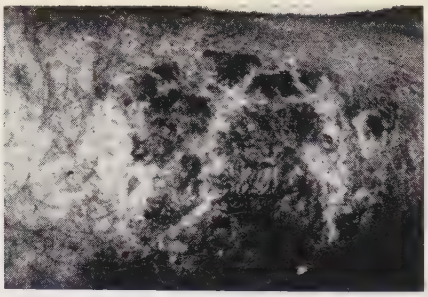

FIGURE 2

Showing decrease in inflammation after 3 days' treatment with griseofulvin.

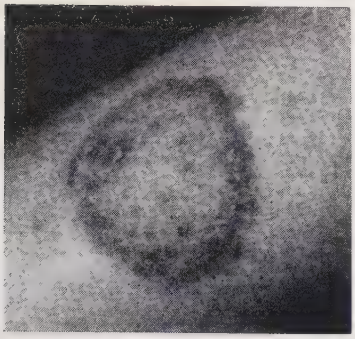

FIGURE 3

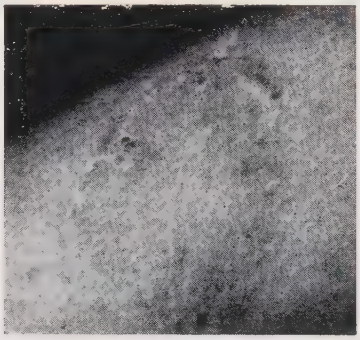

FIGURE 4

Showing increase in size of lesions despite treatment, but decrease in inflammation with less redness and less scaling after 7 days' treatment.

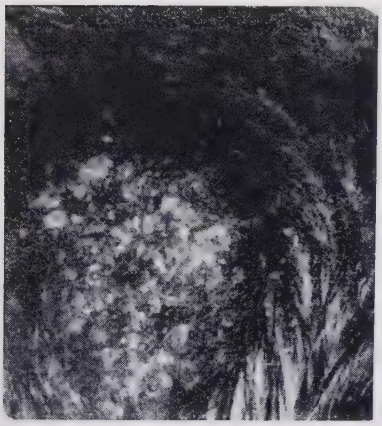

FIGURE 5

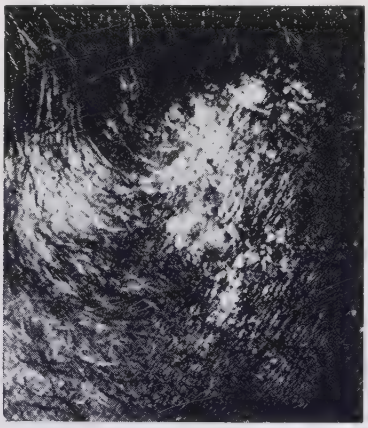

FIGURE 6

Showing healing of kerion of neck after 14 days' treatment.

FIGURE 7

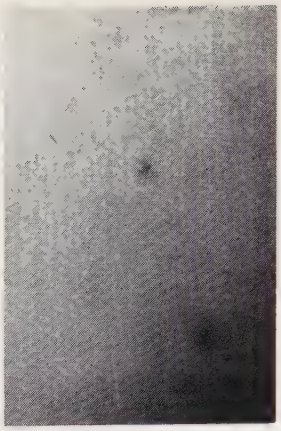

FIGURE 8

Showing relapse 14 days after cessation of treatment—3 fresh papules on sites of healed lesions.

3. There is no significant difference between the two fungi in their response either to griseofulvin (0.8 P 0.7) or to the older treatment (0.3 P 0.2).
4. The severity of the infection is reduced, the shortened course allowing less time for the fungus to invade the deep follicles and so to produce a kerion.
5. Hypertrophic scarring can be prevented if the formation of kerion can be prevented.
6. Relapse occurred 14 days after the drug had been stopped in two cases that were clinically cured.
7. The speed of cure is not significantly related to the duration of the infection before treatment.
8. We suggest that the mode of action of the "curling" factor in griseofulvin, which appears to produce distortion and arrest of growth *in vivo* of the hyphal tip, may be identical with the action of the host tissue factors responsible for terminating the infection in spontaneously regressing lesions.

ACKNOWLEDGEMENTS

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A TRIAL OF GRISEOFULVIN IN TINEA IMBRICATA

R. MacLennan.

Tinea imbricata is common in the Pacific islands and the first reports of the disease were from this region. The first medical description and illustration of the disease were by Alibert (1832) who described a case from New Guinea. Later Turner described it in Samoa (1870), where the local name for it was Lafa Tokelau or Tokelau Ringworm because the disease had recently been introduced there from Tokelau or Bowditch Island. Manson (1879) called it *Tinea imbricata* because of the imbricated arrangement of the scales. Today it is common in all coastal areas of New Guinea, especially in Maprik in the Sepik District, where about 5% of the population have the disease, with important social consequences; for example, a woman with the disease has little chance of marrying a young man; while a man has difficulty in becoming important in the political and magical life of the community, and is often the subject of derision by unaffected members of his village.

In this trial 25 adults and children from Maprik were commenced on treatment at different levels of dosage. All had the typical skin lesions of *Tinea imbricata*—the concentric circles of scaling adherent peripherally with the free borders towards the centre. Many who had had the disease for many years also had diffuse scaling as well. Pruritis occurs at intervals and is mild in contrast to the intense symptoms described by Castellani (1934). Depigmentation of the involved skin may be striking, especially when bordering normal skin. The scalp was involved in only one case. Among 17 subjects, 15 had flattening of the fingerprints so that it was impossible to take a clear print with the standard method; 4 had extensive changes in the nails of the hands or feet, consisting of thickening, ridging or breaking, while 10 had minor changes with slight transverse ridging of their nails. A feature of *Tinea imbricata* which does not seem to have been previously described was marked transverse parallel ridges when the disease involves the skin of the antecubital fossa; it was present in every one of 14 such patients.

Treatment was given in daily doses ranging from 4-24 mg. per kilogram of body weight. Twelve subjects received more than 15 mg./kg. for 4 weeks. Others were treated for much shorter periods, due to lack of co-operation or failure to obtain additional supplies of griseofulvin when needed. With treatment, scales disappeared rapidly at all levels of dosage in 10 to 14 days. Among those treated for a month, little effect was seen on fingerprints, on the nails or on the antecubital ridging. Pigmentation returned to normal in all except one case in which it was returning.

Recurrence occurred eventually in all patients. Among those who had a month's treatment, 8 recurred within 12 days, but could be given further treatment on reduced dosage for another 10 days only and again recurred. Early recurrences were multi-focal, suggesting relapse rather than reinfection. These lesions were as described by Manson in inoculation experiments, and commenced as a papule which enlarged to form a vesicle which dried, in its centre another lesion commenced, and so eventually the typical concentric appearance is produced. Later recurrences were over a month after treatment and tended to follow a visit to a village; the lesions had only one focus at first, suggesting reinfection (Schofield, personal communication, 1960).

Recently in this area Schofield found a significant correlation between low height and weight and *Tinea imbricata* in adults, and suggested that poor nutrition during the growing period as shown by low height, may predispose to the disease. However,

most people with the disease admit having had it since childhood and perhaps this could lead to poor development either from its physical effect which seems to be mild, or as a result of social discrimination with its possible effects on diet. The problem of constitutional predisposition to the disease is important in the consideration of *Tinea imbricata* as a public health problem, since patients can be removed only temporarily from their usual environment.

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DISCUSSION

THE CHAIRMAN: These four papers are now open for discussion.

DR. BROUGHTON: What is the long-term clinical result of this drug? Do all the nails go back to their original state after finishing the treatment? Also, do the *T. rubrum* infections of the skin relapse similarly?

DR. BARLOW: As regards surgical avulsion of the toe-nails combined with griseofulvin, I have done a limited number of cases and they have done extremely well; added to which it has not been quite so incapacitating as seems to have been thought this afternoon. Generally speaking, patients have only been in hospital a few days, and one lady in particular where I did not take one questionable nail off now demands to have it removed. I was very interested in the removal of nails with a dental drill, because the rate of growth of the nail is affected by the removal of the surface. Apparently nail-biter's nails grow approximately four times as quickly as those of the ordinary person. You cannot bite your toe-nails very well, but what you do with dental drills is obviously an alternative.

DR. VAN STEENBERGEN: I quite agree with Dr. Jeremiasse that nails should be removed when treated systemically with griseofulvin, but instead of using abrasion we have weakened the nails with ten per cent barium sulphide after a fortnight of griseofulvin treatment and have repeated it—just as he did with his dental drill—once a month. We have only a very small series, but we are quite content with this simple method that does not require any apparatus.

DR. EVERALL: We have had occasion to remove the toe-nails and finger-nails from six patients, and in one patient after six months the finger-nails are as bad as they were when we started.

DR. C. H. WHITTLE: I hope people will not go rushing to take these nails off before we have found out what is happening to them. I think there are some very interesting things happening.

I would like to ask the members whether it is a common occurrence for them to find other organisms settling in the griseofulvin-treated nails. I have had that experience in one or two cases, and I think the invader is a *Candida*, which can cause persistence of a damaged and broken-up nail.

DR. SHARVILL: I think Dr. Williams has followed some of these nail cases longer than other people. I started treating them in October 1958, and shortly after I started it was felt by Glaxo Laboratories that long-term treatment might be dangerous and we stopped treating them after four or five weeks. I observed that they did continue to improve, and the nails of those early cases cleared quite normally in four to six months, but relapsed at periods of ten to twelve months. This was two years ago. Those results were quite as good as patients who had continuous treatment for six to twelve months and subsequently relapsed after four to six months.

I think one can find other fungi in nails which have been treated, apparently successfully, with griseofulvin, in nails which clear normally and then appear to go wrong, and in nails which become quite normal but then develop discoloured streaks from the base. There is a variety of fungi other than the evident parasites. One may grow *Scopulariosis* from these nails or constantly and repeatedly isolate a fungus which is considered to be a contaminant. I have had patients with a *Trichophyton rubrum* infection and, on many occasions, it has been possible to recover from their nails a persistent species of *Penicillium*.

I would also like to add to Dr. Whittle's remarks about the zoophilic ringworm. I have treated fifteen culturally-proved cases of *Trichophyton verrucosum* with griseofulvin. Using retrospective controls I found that the infection cleared more rapidly than with the conventional treatment.

Dr. Calnan has pointed out that we are often treating an additional allergic reaction, which can be helped by radiotherapy and justifies the use of steroids.

Dr. La Touche: I am very interested in what Dr. Whittle has said about finding *Candida* in the nails of patients treated with griseofulvin, because that is exactly our experience in Leeds. We are finding a great number of these and, in fact, we have got a special pigeon-hole in which we pop the tubes at the end, or towards the end of the resolution of the nail after treatment with griseofulvin. There is a little bit of thickening, or a little bit of discolouration there. We cannot find any dermatophyte, but only *Candida.* We have been treating a number of these cases with Nystatin and they seem to clear up on that.

Dr. Whittle: How did you give the Nystatin?

Dr. La Touche: Topically, as an ointment.

Dr. Wilkinson: I should like to ask Dr. Jeremiasse, with regard to the abrasive technique, whether he takes special precautions against infection and the spread of the infected nail dust that must occur to some extent, and how he sterilizes the instrument. Before the days of griseofulvin when we tried this on some cases we got rather worried, and so did the chiropodist who was doing it. Possibly we got too worried, because it is obviously not everyone who is going to catch *Trichophyton rubrum* in this way, but it is important to be able to assure one's patients that one is not, in fact, spreading any further infection by this technique.

There is one question I should like to ask Dr. Peterkin, and that is whether he has noticed any difference—or whether other members have—in the rate of healing of finger- and particularly toe-nails as between men and women. I think it is far more common for women to have very cold feet and toe-nails, and I wonder whether a vascular effect is relevant.

Dr. Forman: I must say that the early promise of the cure of *T. rubrum* infection in the nails seems to be slowly evaporating. Now we have these techniques for the removal of the nails, which were considered so unsatisfactory in the past. The removal of nails for *T. rubrum* infection only resulted in twenty per cent cure. I think we still need to know what residual infection we are going to find with griseofulvin alone. My feeling about nails from patients treated with griseofulvin is that you may find fungus in nails which are clinically normal, which do not show the slightest sign of thickening, or in nails which are just yellow but apparently structurally normal. Again, you may find fungus in skin on parts of the hand which appear quite normal, and there may be positive scrapings from the toe space when the nails are free of gungus. I think we must allow a period of observation before we can assess the cure and the relapse rate in *Trichophyton rubrum* infection of the nails, treated with griseofulvin. We need to know the percentage of clinical and microscopical cures following griseofulvin treatment, and the percentage of cases of cure of the nails but persistence of fungus elsewhere.

To remove the nails at the commencement of treatment indicates a lack of confidence in the ability of griseofulvin to affect, permanently, a cure on its own. If fungus persists elsewhere, *i.e.* in the toe webs, palms, and soles, then the new nails may be reinfected, after removal.

I would like to refer to the possible sensitising or toxic potentialities of griseofulvin, for it must be remembered that it is being and will be used by doctors under conditions in which careful control of the patient is not easy. Griseofulvin is being given to cases of eczema of the feet and hands, where fungus has not been demonstrated or even when

no attempt is made to prove a fungal etiology. Dr. Calnan has referred to leucopenia occurring in children. If in any individual case the leucopenia persists, then the administration of the drug should be stopped.

Nausea and headache, requiring the reduction of the dose to half a gramme a day in adults, have been encountered. Two patients of mine complained of temporary pins and needles of the hands and it would be remarkable if other manifestations of sensitisation or toxicity do not appear.

PROF. VANBREUSEGHEM: I wonder if anybody has paid attention to the age of the patients with nail infections so far as the rapidity of the cure is concerned. From my own observations, I would actually discourage people of more than thirty years from being submitted to treatment by griseofulvin for nail infections. On the other hand, I feel that under thirty years they are cured more easily. Has anyone else made such observations?

DR. PETERKIN: Dr. Wilkinson asked if men and women are different in regard to nail growth. I would say the more important factor was youth rather than sex. With a young woman the nails would heal fast, and a young man's nails would heal fast. I have seen one or two cases of husband and wife or father and daughter, and it was not the sex that made the difference but the age.

THE CHAIRMAN: Would any of the speakers like to reply to any specific questions?

DR. PETERKIN: May I deal first with the long-term clinical results. The time is short, as yet, and we cannot really assess them. I know the impression I have is that finger-nails will often keep well, and palms and even soles with *Trichophyton rubrum* will, but toe-nails are a very different matter indeed. With regard to avulsion, one has often used it before. I have only had three cases treated with avulsion and griseofulvin, but I have not had to have any of these in hospital at all; they can be done perfectly well in our out-patients' department, and they seem to walk about perfectly well. So there is no need to feel that you are suggesting the patient should do anything serious.

DR. JEREMIASSE: With regard to the first part of Dr. Wilkinson's question, the dental drills are sterilized in the same way as all the surgical instruments. We do take precautions after abrasion; the skin is treated with iodine. Theoretically, precautions are not necessary because the mycotic dust falls on keratin which is already made resistant by the orally-administered griseofulvin.

SPECTROPHOTOFLUOROMETRIC ASSAY OF GRISEOFULVIN IN BODY FLUIDS AND TISSUES

E. G. Tomich and K. J. Child

Some eighteen months ago, with our colleague Dr. Muggleton at Glaxo Laboratories, we decided to investigate the metabolism and distribution of griseofulvin in laboratory animals and in man. We planned to study blood and urine levels in human volunteers given single oral doses, and tissue and body fluid levels in animals treated orally or parenterally. Later we conducted some *in vitro* incubation studies to locate the organ or organs responsible for the *in vivo* catabolism of griseofulvin.

To carry out these studies we needed a rapid and reliable method of assay. At that time Dr. Muggleton and his associates had already developed a microbiological tube assay with *Microsporum canis*, but this had severe limitations. For example, it took at least three days to give a result, and samples had to be sterile. Further, griseofulvin could not be assayed by it in whole blood. For these reasons we sought an alternative method of assay, and found it while investigating the characteristics of the antibiotic in a spectrophotofluorometer. This instrument, which was developed by Dr. Robert Bowman and Dr. Bernard Brodie at the National Heart Institute in Maryland, U.S.A., enables one to activate solutions at any required wavelength between 200 and 600 mμ and to measure the resultant fluorescence at any wavelength in the same range. Thus, provided a substance can be isolated from fluids and tissues and will then fluoresce in ultra-violet or visible light, it can be assayed by a fluorometric method.

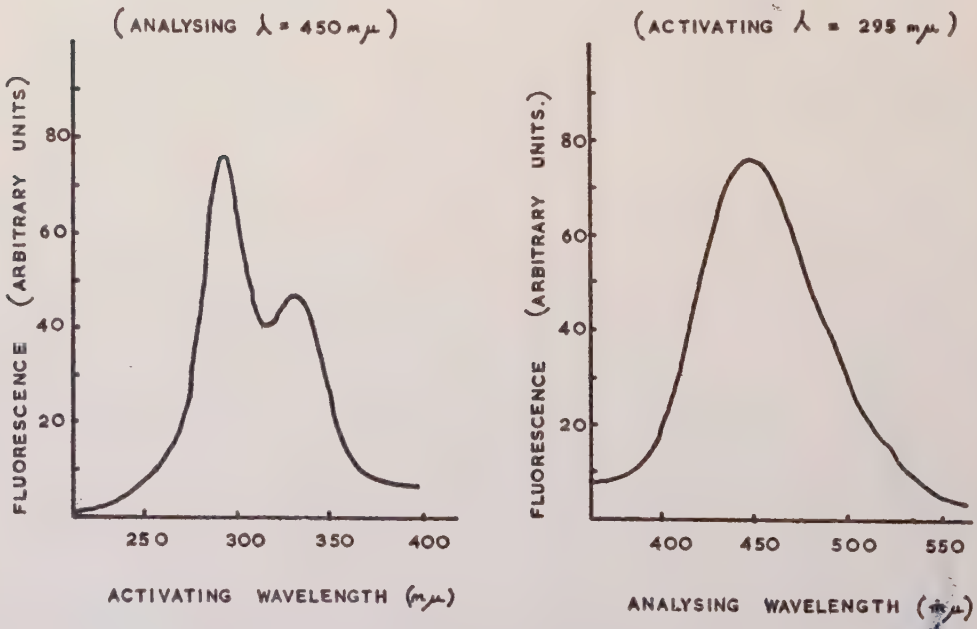

Fig. 1. Fluorescence spectra of griseofulvin. See text.

A 1% ethanolic solution containing 0.5 μg griseofulvin/ml. was scanned on a spectrofluorometer, and Fig. 1 shows the spectra obtained. On the left is the activating spectrum obtained at an analysing wavelength of 450mμ. It shows two peaks, one at 295 mμ and a smaller one at 335 mμ. The right-hand curve, obtained by activating at 295 mμ and scanning the fluorescent spectrum, shows only one peak, and that is at 450 mμ. For assay purposes, therefore, solutions are activated at 295 mμ and analysed at 450 mμ.

The fluorescent activity of an aqueous griseofulvin solution is independent of pH over the range 3 to 10, but below pH 3 activity falls off rapidly. We have not studied the effects of pH values above 10.

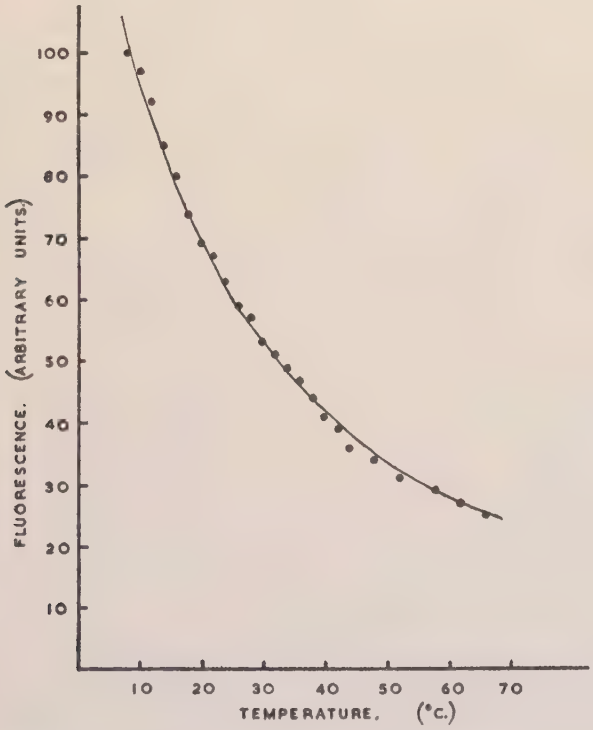

Fig. 2. The effect of solution temperature on the fluorescence of griseofulvin.

Test and standard solutions must be compared at the same temperature. Fig. 2 shows the effect of heating a solution of griseofulvin and measuring its fluorescence at various temperatures. Over the range 10° to 25°C. the decrease in activity with increasing temperature is linear. A loss in activity of about 40% occurred when the solution temperature was raised from 10° to 25°. Full activity was restored by lowering the temperature to its original value.

Before describing assay procedures, there is one further point whose importance cannot be over-emphasised. All apparatus, syringes, flasks, samples-tubes, shake-tubes, pipettes, cuvettes and so on must be scrupulously clean. All apparatus is cleaned with

chromic acid and distilled water, washed with 1% ethanol, and the washings are then checked for fluorescence. No apparatus can be considered fit for use until it has been subjected to this test.

To assay heparinised whole blood, plasma or serum, a small sample, 1 ml. or less, is shaken for 15 seconds with 10 ml. ether, and 8 ml. of the ether phase are then removed and evaporated to dryness on a water-bath. The residue is dissolved in 10 ml. of 1% ethanol, and the fluorescence of this solution is then compared with that of a standard griseofulvin solution. The recovery of griseofulvin from blood, plasma or serum is about 96%. Recently we have modified this assay by using smaller volumes and micro-cuvettes, so that it is now possible to assay as little as 0.1 ml. blood.

Urine contains fluorescent substances, but fortunately they are acidic in nature. If the pH of the urine sample be raised to about 10 before the ether is added, the interfering substances will remain in the aqueous phase. Recovery of griseofulvin from urine treated in this manner is about 90%.

Tissues may be assayed by finely dividing 1 gm. samples with scissors and refluxing each with 10 ml. ether for 30 minutes. After filtering off the ethereal solution, washing the residue with a further 10 ml. ether and again filtering, the combined extracts are evaporated to dryness and the residue is shaken with 10 ml. of 1% ethanol. The suspension is filtered to remove undissolved fat, and the filtrate is assayed on the spectro-

Tissue	*Mean percentage recovery ± standard error*
Heart	89.0 ± 7.6
Lung	61.0 ± 4.2
Liver	36.0 ± 2.9
Spleen	84.0 ± 6.4
Kidney	66.0 ± 4.2
Testes	70.5 ± 3.8
Skin	26.0 ± 2.5
Skeletal muscle	44.0 ± 2.9
Smooth muscle	74.0 ± 7.7
Fat	16.5 ± 2.1

TABLE I

Recovery of griseofulvin added to finely divided rat tissue. The values shown are the means of twelve recovery experiments.

fluorometer. Table I shows the percentage recoveries obtained when griseofulvin was added to, and then isolated from, various rat tissues. It is not surprising that the recoveries were highest from the tissues with the lowest fat contents.

We have compared the *Microsporum canis* tube assay with this spectrofluorometric procedure. Human volunteers were given single oral doses of 0.5 gm. griseofulvin, and blood samples were taken by venipuncture at intervals over the next 24 hours. The samples were allowed to clot, and the sera were assayed for griseofulvin by both methods. Fig. 3 shows the results. The two concentration-time curves are almost identical. Further, since the microbiological assay measures total anti-fungal activity and the spectrofluorometric assay is specific for griseofulvin, it follows that the only anti-fungal compound circulating in the bloodstream is griseofulvin itself.

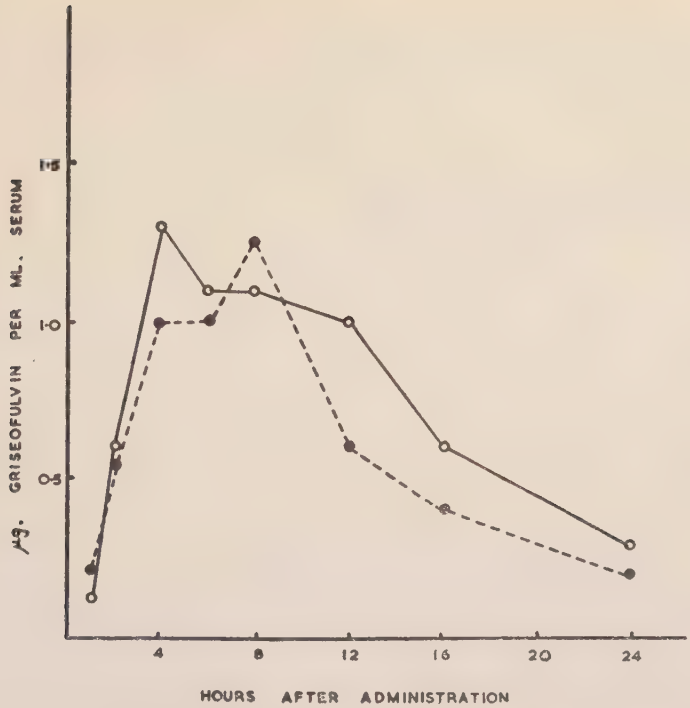

Fig. 3. Serum levels of griseofulvin in man after a single oral dose of 0.5 gm. Each value represents the group mean for three individuals.

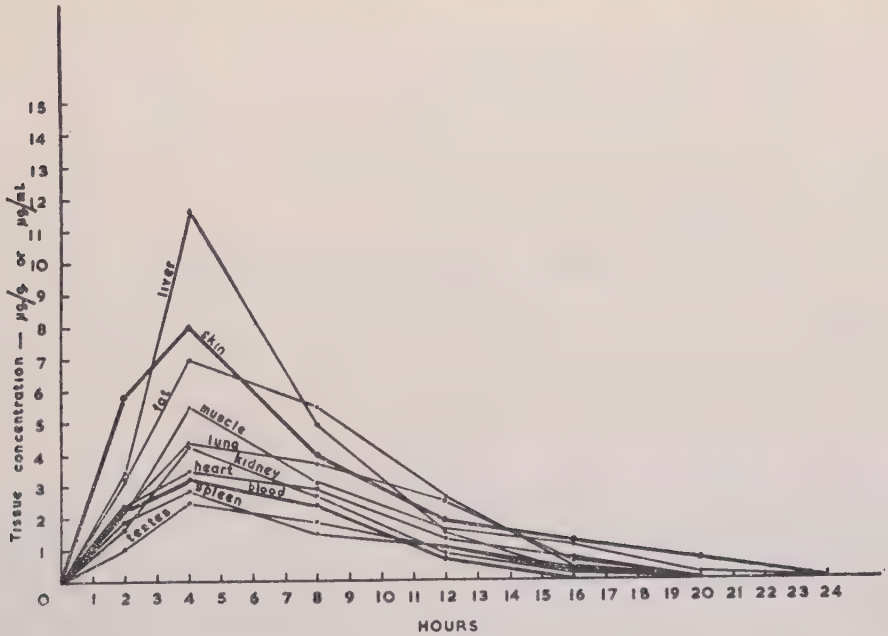

Fig. 4. Tissue griseofulvin levels in rats given single oral doses of 100 mg./kg.

Fig. 4 shows various tissues levels in male rats during the 24 hours immediately after receiving single oral doses of griseofulvin at 100 mg./kg. The tissues examined were blood, shaved whole skin, liver, omental fat, skeletal muscle, lung, kidney, heart, spleen and testis. Peak concentrations were recorded at 4 hours in all tissues. However, the blood concentration was zero at 16 hours; but the whole-skin levels persisted for a further 8 hours. We were not able to assay griseofulvin in keratin as distinct from whole skin, so that we cannot be sure that some griseofulvin, either free or bound, was not still present in the keratin at 24 hours.

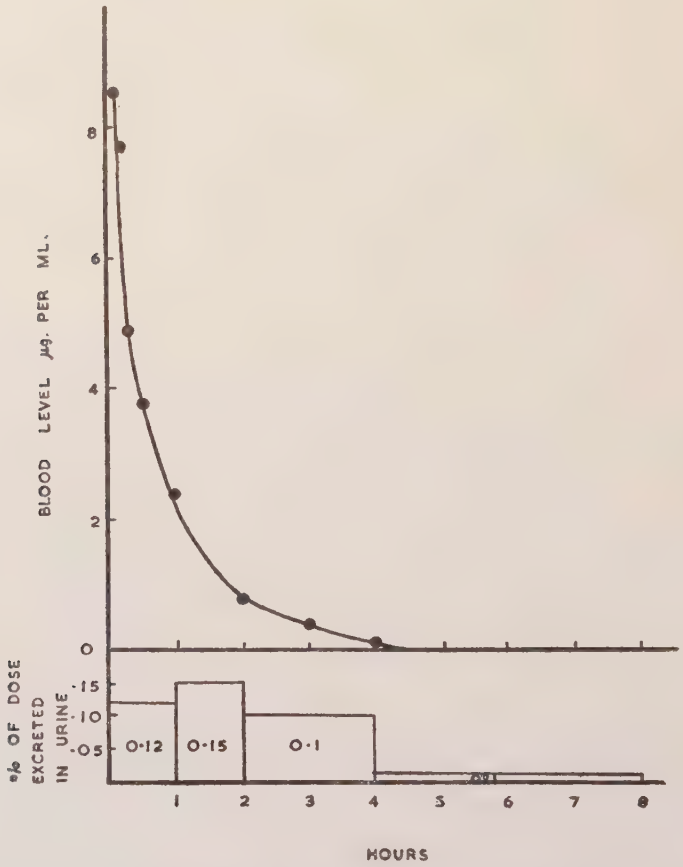

Fig. 5. Blood and urine griseofulvin levels in rats given single intravenous injections of 20 mg./kg.

Fig. 5 records the blood and urine levels in male rats injected intravenously at 20 mg./kg. with griseofulvin dissolved in 75% N-N-dimethylformamide. It should be noted that, although this solvent is most useful for dissolving griseofulvin to administer to laboratory animals, it is not recommended for intravenous clinical administration. The blood level falls precipitously: at least 90% of the dose disappears within 5 minutes, indicating a rapid distribution into total body fluid. Less than 0.5% of the

dose was excreted as unchanged griseofulvin in the urine within 8 hours of injection. Similarly, we have never found after oral dosing more than 0.6% of the dose in the urine of either rats or man, even over 24 hours.

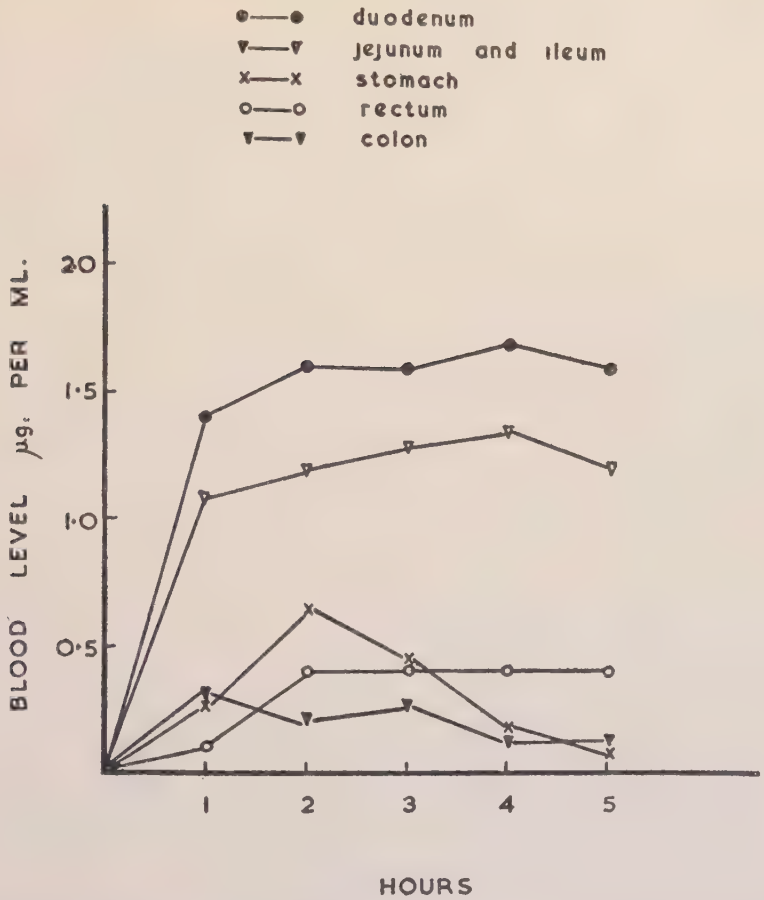

Fig. 6. Blood levels of griseofulvin in cats after direct administration of 100 mg./kg. at various sites in the gastro-intestinal tract. Each point is the mean value for 3 cats.

We have attempted to determine the site or sites of absorption of griseofulvin by injecting the antibiotic directly into various ligated segments of the gastro-intestinal tracts of anaesthetised cats and then following the peripheral blood concentration-time curves. Fig. 6 shows that under these conditions absorption was most efficient from the duodenum, with that from the jejunum and ileum coming a close second. Some absorption occurred from the stomach and even some from the rectum. After being administered directly into the large intestine, griseofulvin was detected in the blood-stream, although the levels there were low and barely measurable. The results indicate

that, in cats at any rate, griseofulvin is absorbed along most of the gastro-intestinal tract. Should this occur in man also, it would explain the prolonged blood levels that result from a single oral dose.

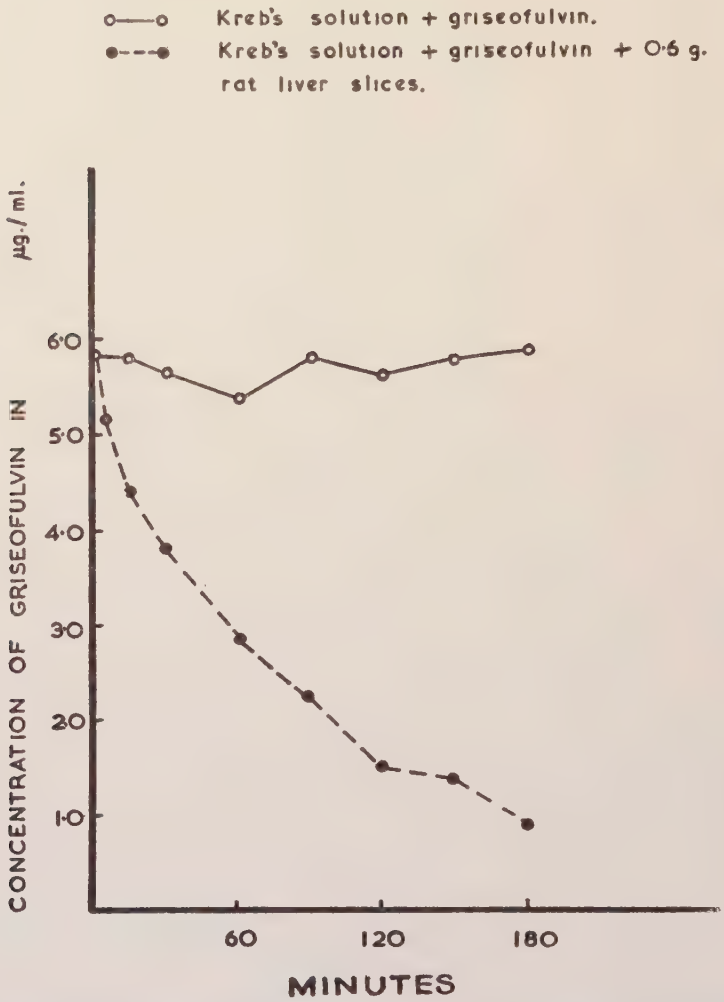

Fig. 7. Metabolism of griseofulvin by rat liver slices at 37° C. incubated in Krebs bicarbonate solution oxygenated with 90% O_2 + 5% CO_2 at pH 7.4. Results expressed in µg./ml.

Fig. 7 illustrates the results of incubating rat liver slices with griseofulvin in oxygenated Krebs solution at 37°C. The upper line represents the control solution, and the lower line the solution containing the liver slices. We have examined kidney, spleen and lung slices under similar conditions, but have been unable to detect any destruction of griseofulvin by these tissues

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NAILS AND GRISEOFULVIN

R. Vanbreuseghem

This paper presents two different aspects of the relations existing between nails and griseofulvin.

The first is the therapy of ringworm of the nails by griseofulvin. Everyone knows this problem but many do not realise its complexity or its difficulty. Too many papers have presented as a cure what was only an improvement, and too many people do not understand that, even in the best cases, what we have done is not cure the patient but oppose a provisional barrier to the growth of the dermatophyte.

The second aspect of the relation between nails and griseofulvin is probably less obvious. It is a common observation that the distribution of ringworm of the nails in a patient is very irregular: one nail only may be invaded whereas the others remain intact, two nails situated quite apart may be attacked; the nails of one hand may be invaded and the nails of the other hand may remain absolutely "immune"—if that is the right way to express the fact that some nails of the same patient appear absolutely resistant to the attack of dermatophytes. On the other hand, it has been reported occasionally that some nails may heal spontaneously although the others remain infected. It is difficult to express all the possibilities of this question, but I would add that it is well-known that the nails invaded by the dermatophytes do not present the same degree of invasion, their resistance to the attack of the fungi being, obviously, absolutely different. I do not think that a sound explanation of these facts has ever been produced. During the treatment of nails by griseofulvin it has also been recognised that some nails improve more rapidly than others, and it has been generally admitted that toe-nails are not so easy to cure as finger-nails: this has been generally explained as a result of the slow growth of the toe-nails compared with the rather rapid growth of the finger-nails. In the second section of this paper I will try to demonstrate that, independently of the variations in their "natural" disposition to heal spontaneously or when submitted to some form of therapy, the cure of the nails during treatment by griseofulvin could be greatly dependent on the fact that griseofulvin does not enter every nail of the same hand or of the same foot with the same ease. This could explain why every nail of the same patient would not heal with the same facility, and why in some cases the treatment is a complete failure. If my observations are confirmed—and I insist on the fact that they require confirmation on a much larger basis than my preliminary experimental work recorded here—it would indeed make the prognosis of treatment still more complicated than it now appears.

PART I

TREATMENT OF ONYXIS DERMATOPHYTICA BY GRISEOFULVIN

The use of griseofulvin for the treatment of ringworm of the nails is obviously one of its most important applications. Whereas we already have different types of treatment for *tinea capitis* as well as for *tinea corporis*, nobody can pretend that he had a method to cure ringworm of the nails. For this reason I concentrated on the treatment of onyxis by griseofulvin and by griseofulvin alone, though I am not convinced that it was the surest way of obtaining the most satisfactory results. It is actually not impossible that some of our old methods of treatment, by topical applications or by surgery, could achieve better results when combined with griseofulvin than those results obtained by griseofulvin alone.

I have treated 7 patients, all of them presenting onyxis caused by *Trichophyton rubrum* and some skin lesions of the same origin. They were all adults and had been infected by *T. rubrum* for 2 to 14 years. Griseofulvin was given by mouth in a dosage

of 2 gm. a day for the first month and, usually, in dosages of 1 gm. a day for the subsequent months. The treatment was continued for 6 to 8 months, and some of the patients are still under treatment. Five of the 7 patients improved, and 2 look to be completely cured. Considering the fact that these patients had been treated, often for years, with the most "modern" drugs without success, the result is more important than it looks. But compared with the patients with *tinea capitis* who respond so regularly to griseofulvin, in a minimum time with a maximum of safety, patients with ringworm of the nails are confusing because of the irregular evolution of the disease, their slowness, if not to improve, at least to heal, and sometimes by the side-effects produced by the drug.

If we exclude the modifications of the blood picture, only one of our 7 patients presented some side-effects: headache, anorexia, insomnia, loss of weight and a distressing urticaria that was relieved by reducing the dose of griseofulvin. But nearly every patient had some alteration of the differential leucocyte count with a slight leucopenia and monocytosis. In one patient the white cells dropped to 3,400 per cubic millimetre. I have also observed, towards the 5th or 6th month of treatment, a slight anemia with a reduction of the red blood cells and of the haemoglobin. One patient, the same one who had the 3,400 white blood cell count, had 0.9% erythrocytes with punctate basophilia, which at this level is considered to be a manifestation of some intoxication. The basophilic erythrocytes dropped to 0.3% after treatment with liver extract. The alteration of the blood regresses usually after some time with or without medication, and except in very severe cases is not an argument against the use of griseofulvin.

It does not seem that the duration of the treatment can be less than 6 months. However, the time required to cure ringworm of the nails is probably very variable from one patient to another. In my very short series the two cures were obtained in patients less than 30 years old. I did notice that a young man, 23 years old, improved much faster than the other ones. I am at present of the opinion, but without statistical support, that the age of the patient is of greater importance than the age of the disease: I mean that a young patient has the best chance of cure, even if his disease is nearly as old as himself. The youngest of my "cures" was 28 years old and infected for at least 14 years.

Besides the common observation of the slowness of improvement of the toe-nails, some nails improved much more slowly than others. This fact may be dependent upon those unknown factors which make some nails more resistant than others, or which are the basis for their spontaneous cure. But as we will see in the second section of this paper, they may depend not only on the quantity of griseofulvin given by mouth, but on the quantity of griseofulvin which gets into the nails. I have observed in some cases that nails during treatment by griseofulvin become harder and thicker. I have no explanation of this phenomenon, which once more reflects the diversity and versatility of human nails. It is also evident that a clinical cure is compatible with a state of mycotic infection, and a complete cure cannot be claimed without mycological verification.

Below is a short description of the patients and their response to treatment.

Case 1 D.M., male, aged 45.

Two years ago the nails of the right hand were removed surgically but every one regrew infected. He had bilateral athlete's foot and ringworm of the groin. *Trichophyton rubrum* was cultured from the foot and from the inguinal region, but never from the nails although mycelium was observed in scrapings of them. Different topical treatments improved the situation, but not a single nail was cured. From June 1959 to February 1960, the patient received 270gm. griseofulvin: 2gm. a day for the first month, 1gm. a day later on. After 3 weeks of this treatment, there was nothing more to see of the skin lesions. The nails also improved and even healed, with the exception of the nail of the fourth finger of the right hand which is still infected at the completion of the treatment. The drug was tolerated very well. A slight leucopenia was observed around the third month and by the sixth month a hypochromic anemia was relieved by a mixture of vitamin B 12, folic acid and iron. The last infected nail was quite abnormally thick and hard, and is unchanged in April 1960.

Conclusion: Drug well tolerated. Moderate modifications of the differential blood count and slight anemia. Cure of the skin lesions and of some nails but one nail is not influenced by the treatment.

Case II. K.M., female, aged 30.

Athlete's foot and onyxis of the IV and V left toe nails for 12 years. The real cause of the disease had not been ascertained until more than 8 years ago and from that time on different medications have been used with, from time to time, slight improvement. For the last 8 years. *T. rubrum* has been isolated at least once a year from the skin or nails. In July 1958 ringworm of the right palm, from which *T. rubrum* was cultivated. Griseofulvin was given from 17 June 1959 to 17 February 1960: 2gm. a day for the first month, 1gm. a day later on. Between 17 February and 16 March 1960 the patient took twice, at 15 day intervals, a single dose of 4gm. The total dosage of griseofulvin administered amounted to 278gm. On 17 February 1960 the patient looked clinically cured, but T. rubrum was again cultivated from the nail. However a new culture on 16 March was negative.

This patient is the only one who has manifested side effects of any importance: nausea, feeling of tiredness, anorexia, insomnia, headache. Five days after starting the griseofulvin therapy, development of urticaria especially localised on the external surfaces of the limbs and associated with intense dermographism. However, after 15 days of treatment the skin lesions were considerably improved. The griseofulvin was reduced to 1gm. a day and simultaneously 3 x 50mg. of Benadryl were given daily for a week: urticaria vanished rapidly after this modification in the treatment and did not reappear. However the other side effects persisted as long as the patient recieved griseofulvin. The blood was regularly tested and the differential blood count showed a monocytosis with a leucopenia of 3,400 white cells per cubic millimetre 2 months after the beginning of the treatment. The total of white cells was 4,300/cu.mm. after 3½ months, but 3,700/cu.mm. at 4½ months. At the same time there was a slight anemia; at its lowest 3,800,000 red cells and 71% hemoglobin. On 12 March 1960 the white cells were 6,000/cu.mm., the red cells 4,050,000 and the Hb. 80.5%: everything looked practically normal but erythrocytes with basophilic granules accounted for 0.9%, a rate considered by most experts a proof of toxicity. Liver extract was given, 6 injections of 1.5 ml. of crude liver extract (Lilly), and the next month the erythrocytes with basophilic granules had dropped to 0.3%. From August 1959 there was, without interruption, some slight anisocytosis which in February 1960 became a true anisocytosis. Actually the appearance in the blood of the erythrocytes with basophilic granules immediately followed the administration of a single dose of 4gm. griseofulvin — 4 x 1gm. in 24 hours; the ingestion of such a large quantity of the drug produced slight lipothymia, headache and nausea for 3 days. It should be noted that when this 4gm. dose was given griseofulvin had been stopped for 15 days. Another 4gm. dose, 15 days after the first one, was well tolerated, except for a redness of the face.

Conclusion: Clinical cure after 8 months, mycological cure after 9 months. Side effects: urticaria, headache, anorexia and insomnia. Blood alterations: leucopenia, anemia and 0.9% erythrocytes with basophilic granules. Disappearance of the symptoms of toxicity by suppression of the drug and liver therapy.

Case III. E.L., male, aged 60.

Onyxis of the right hand 12 years ago. Radiotherapy 3 years ago to cure the onyxis: complete failure and radiodermatitis of the right hand. On 8 October 1959 slight onyxis of every toe nail and onyxis of all nails of the right hand which are very thick. No lesions of the nails of the left hand. Tinea of the neck and of the right ear. *T. rubrum* was cultured from those lesions or seen in the scrapings. The weight of the patient is 100 kg. (220.46 lbs.). During the first 5 months he took 2gm. griseofulvin, and later on 1gm. a day. The skin lesions disappeared in the first 15 days, and after 6 months the nails were greatly improved. However, the toe nails were much more improved than the finger nails. No side effects. Very slight leucopenia at the 5th month. Cultures made at the 5th month have all given negative results, and mycelia have been seen in only one scraping in four.

Conclusion: Healing of the skin lesions in 15 days, improvement of the nails in 6 months, no side effects.

Case IV. S.D.W., female, aged 40.

The correct diagnosis of the disease had been made 3 years ago, but symptoms developed 13 years ago for the first time and were considered as eczema. On her first visit 3 years ago there were lesions of the nails, soles of both feet and lower half of calves where they had a granulomatous aspect. *T. rubrum* was easily cultured. A very disturbing palmar dyshidrosis was rapidly improved by the treatment of the foci by different fungistatic drugs used locally. At the beginning of the treatment with griseofulvin every toe nail was completely invaded, whereas of the finger nails the I and III of the left hand are the only ones that are affected, and for only half of their length. The soles are still hyperkeratotic but the calves are cured.

From 26 June 1959 to 3 March 1960, 2gm. griseofulvin a day for the first month, 1gm. later on. The total dosage was about 300gm. After 15 days of griseofulvin the skin lesions looked clinically cured, but the improvement of the nails was much slower. On the last examination (3 March 1960) the fingernails were cured clinically and mycologically. On both feet the nails IV and V looked cured, II and III greatly improved, but the I was practically unchanged. A scraping of the toenails gave a culture of *T. rubrum*. No side effects except

for slight monocytosis around the third month, a slight hypochromic anemia at the fifth month and a reduction of the red cells at the seventh month.
Conclusion: Cure of the skin lesions in a very short time, improvement of the onyxis with cure of the fingernails, no subjective side effects, slight monocytosis on the third month, hypochromic anemia on the fifth and seventh month.

Case V. F.M., male, aged 23.
He was one of a group of young sailors who, in the Persian Gulf, were the victims of a *T. rubrum* epidemic. Actually he is the only one I have seen: widely disseminated ringworm from which *T. rubrum* was cultured on 29 September 1958. Improvement by local therapy, but during the treatment a left fingernail was invaded. One year later the right fingernails I, III and V were invaded. *T. rubrum* was again cultured. From 15 September 1959 to 4 November 1959, 1gm. of griseofulvin daily: after those 50 days treatment the skin lesions had completely vanished, apparently from the very beginning of the treatment, and the nails were cured over half of their length. No subjective side effects but a very slight monocytosis at the completion of the 50 days treatment.
Conclusion: Very rapid cure of the skin lesions and spectacular improvement of the nails in 50 days with 1gm. of griseofulvin daily. No subjective side effects but slight monocytosis after 50 days treatment.

Case VI. V.C., male, aged 41.
Seven years ago he had already had ringworm for a good many years. *T. rubrum* was cultured at this time and every form of therapy available was a complete failure: radiotherapy, surgical removal of the nails, and local therapy by fungistatic drugs. On 3 June 1959 the right fingernails had been completely destroyed by the dermatophyte, whereas the left fingernails looked absolutely normal. Every toenail was deeply invaded except for the left IV and V nails and the right III nail which looked intact. There was also bilateral athlete's foot and ringworm of the right palm. During the first month 3gm. griseofulvin a day, 1gm. a day for the next three months, and 1.5gm. a day for the last two months. After this treatment — total dosage 240gm. — every fingernail was cured up to 1 to 2mm. from the free edge, but they were thicker and harder than normal. The right toenails IV and V looked cured and the others greatly improved. The skin lesions disappeared in the first 15 days of the treatment. At the completion of the treatment *T. rubrum* could not be isolated from any site where it was looked for. No subjective side effects. Slight monocytosis around the 3rd month and slight anemia in the 5th and 6th months.
Conclusion: Rapid cure of the skin lesions, improvement of the nails, no subjective side effects, slight monocytosis and anemia.

Case VIII. R.V., male, aged 28.
For 14 years onyxis and athlete's foot. Eight years ago every nail was invaded and the skin of the calves and of the groins presented typical lesions of ringworm. There was also a bilateral athlete's foot. All those lesions produced abundant colonies of *T. rubrum*. Simultaneously Pityriasis versicolor and erythrasma. His mother and only brother had very similar lesions, but his father and wife were examined and found normal. Different types of local therapy were used for years with slight improvement but nothing like a cure. On 25 August 1959 griseofulvin therapy was started: every toenail was invaded and the fingernails were destroyed except for the III left nail which was invaded only over half of its length. There was also bilateral athlete's foot and ringworm of the groins and the calves as noted previously. During the first month of griseofulvin therapy the patient took 2.5gm. daily, 1.5gm. the 2nd and 3rd month, and 1gm. during the other months until 11 February 1960. The patient thought that he was better with a dosage slightly higher than the one I had proposed: 2gm. for the first month and 1gm. afterwards. Improvement of the skin lesions from the 8th day and maybe a slight improvement of the nails at this time. After 2 months the fingernails had regrown until 1 to 2mm. from the free edge, and looked cured by the third month. Clinical cure after 4½ months. No mycological examination at this time, but one month later the mycological cure was perfect. The Pityriasis versicolor has not been influenced by griseofulvin. No subjective side effects and no modification of the blood picture.
Conclusion: Complete cure in 5½ months, maybe in 4½ months. No side effects. No influence of griseofulvin on Pityriasis versicolor.

From these seven cases it may be concluded that griseofulvin for the treatment of dermatophytic onyxis gives better results than any previous therapy. However, a cure is difficult to obtain. Out of the 7 patients, 2 have been completely cured, one after 5½ months, the other after 8 months. Every other patient was improved. The dosage has been 2 gm. per day for the first month and 1 gm. a day for the other months. Subjective side-effects have been observed in one patient only, but slight modification of the blood picture (leucopenia, monocytosis and anemia) developed from the third to the sixth month in 5 of the 7 patients.

PART II

PENETRATION OF NAILS BY GRISEOFULVIN

In the introduction I have put forward some arguments in support of the view that griseofulvin given by mouth does not penetrate equally well into every nail, if we judge from results obtained by this therapy. However, current observations indicate that the resistance of nails to the dermatophyte and their natural tendency to heal spontaneously when invaded varies considerably. That invalidates our judgement on the real efficacy of griseofulvin. For these reasons I tried to decide experimentally if every nail is equally susceptible to the penetration of griseofulvin.

Technique

Young white mice weighing about 20 gm. each were fed with a daily dose (1.25 mg.) of griseofulvin. Griseofulvin emulsified in milk was given by mouth. One single drop of the emulsion was estimated to contain about 1.25 mg. of the antibiotic. Griseofulvin was given for periods of 15, 21 or 28 days. At the end of those periods the mice were sacrificed, and the nails were removed by traction with forceps, wrapped individually in paper, giving each of them a number indicating their position as well as the animal from which it was extracted, and autoclaved for 20 mnutes at 115° C.

The nails were then arranged along the centre line of a slide covered aseptically with Sabouraud agar. Each slide was deposited on a U tube placed in a sterile Petri dish. About 3 ml. of sterile distilled water was added to every Petri dish. The dishes were then incubated at 25° C. After 5 days' incubation the slides were observed under the microscope, and the same was done after 10 days. After this last observation, the slides were removed from the Petri dishes, the cultures developed on the under face of the slide—i.e. on the side which faced the bottom of the Petri dish—removed, and the slides dried at 37° C. The nails were then removed carefully and their position on the slide noted. The slides were next dipped in dilute collodion, allowed to dry for a few more days at 37° C., dyed with cotton blue, and examined again. For this technique of Rivalier and Seydel, see Langeron and Vanbreuseghem (1952).

Tentatively, in an endeavour to enlarge the quantity of blood supplying the nailbeds, a drug with vasodilating power on the small arteries of the fingers was given to some animals together with griseofulvin. Without prejudice or experience in this field, I chose a derivative of dibenzazepine, RO 2-3248 or azapetine.* This drug has a long-lasting vasodilating effect and has been submitted by different authors to clinical as well as experimental trial. The quantity of azapetine administered daily to some of the mice was 1 mg. in powder for 4 mice. This powder, enriched with lactose (10 gm. lactose for a tablet containing 25 mg. azapetine) was spread on the surface of two pieces of bread, and 10 ml. of milk were put on the bread. Mice fed with azapetine—given immediately after griseofulvin—did not receive any other food than the bread and milk.

The mice were weighed before and at the end of the experiment; all of them gained some weight (Table I), but the gain was less for the mice fed on griseofulvin and azapetine than for the mice fed on griseofulvin only. The reason for this difference is not clear. Actually, the mice fed griseofulvin received grain, carrots, and salads; on the other hand, the mice fed griseofulvin and azapetine received only bread and milk. But the differences in diet were probably not the only ones; it must be noted that in addition, and in relation to its vasodilating action, azapetine has a diuretic action.

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* Ilidar, from Roche Ltd., to whom I am indebted for the product.

Period of treatment	*Total weight of 4 mice* ***before*** *treatment*	*Average*	*Total weight of 4 mice* ***after*** *treatment*	*Average*
15 days	78.45 gm.	19.6 gm.	102.75 gm.	25.7 gm.
15 days	86.85 gm.	21.7 gm.	99.85 gm.	25. gm.
21 days	71.35 gm.	17.8 gm.	89.71 gm.	22.4 gm.
28 days	78.15 gm.	19.5 gm.	88.77 gm.	22.2 gm.
21 days	84.4 gm.	21.1 gm.	90.53 gm.	22.6 gm.
15 days + Azepine	72.5 gm.	18.1 gm.	75.18 gm.	18.8 gm.
21 days + Azepine	75.3 gm.	18.8 gm.	80.63 gm.	20.2 gm.

TABLE I

Effect of griseofulvin on weight

This diurectic action could be considered as responsible for a greater elimination of griseofulvin and consequently for a reduced quantity of griseofulvin in the nails.

Two strains of dermatophytes were used in these experiments. The first, and the one mostly used, was a strain of *Microsporum gypseum* (RV. 10906) isolated from soil in the Belgian Congo. The second was a strain of *Trichophyton mentagrophytes* (RV. 11053) isolated in Belgium from a case of *tinea pedis*. Since the time they were isolated, i.e. 3½ months before the experiment for *M. gypseum*, and 22 days for *T. mentagrophytes*, the strains were cultured on Sabouraud agar. For inoculation of the slides we used cultures of about the same age. The differences in age of subcultures has not apparently made any difference to the results observed. I will, however, give the age of those subcultures for each series:

Series I	*Microsporum gypseum*	21 days old
Series III		24 days old
Series IV		20 days old
Series VI		16 days old
Series VII		20 days old
Series VIII		20 days old
Series IX		34 days old
Series II	*Trichophyton mentagrophytes*	22 days old
Series V		26 days old
Series X		28 days old

SERIES OF MICE: The number of mice used in each experiment was small, but altogether 802 nails were obtained. The mice were divided into ten series.
Series I: 4 mice fed griseofulvin for 15 days and inoculated with *M. gypseum*.
Series II: 4 mice fed griseofulvin for 15 days and inoculated with *T. mentagrophytes*.
Series III: 4 mice fed with griseofulvin for 21 days and inoculated with *M. gypseum*.
Series IV: 4 mice fed griseofulvin for 28 days and inoculated with *M. gypseum*.
Series V: 4 mice fed griseofulvin for 21 days and inoculated with *T. mentagrophytes*.
Series VI: 4 mice fed griseofulvin and azapetine for 15 days and inoculated with *M. gypseum*.
Series VII: 4 mice fed griseofulvin and azapetine for 21 days and inoculated with *M. gypseum*.
Series VIII: 3 mice, 5 days old, born from one mouse fed 25 days with griseofulvin. Inoculated with *M. gypseum*.

Series IX: 8 mice, no griseofulvin, no azapetine, inoculated with *M. gypseum.*
Series X: 8 mice, no griseofulvin, no azapetine, inoculated with *T. mentagrophytes.*
Microsporum gypseum was the test organism for the series I, III, IV, VI, VII, VIII, and IX, *Trichophyton mentagrophytes* for the series II, V, and X.

Reading of the Slides

The slides were examined three times: after 5 days' growth, after 10 days' growth, and after they had been dried and stained. The first two examinations were made to determine whether or not the nails were invaded by the dermatophytes. The third examination was to determine if the morphology of the dermatophytes, on the slides where nails from animals fed griseofulvin had been used, was different from the morphology of the dermatophytes on the slides with nails from untreated animals.

On the surface of the agar that covers the slide in a thin layer, the mycelium develops beautifully and regularly unless something stops its growth. Of course, this development occurs not only on the upper surface of the slide but also on the under surface as both surfaces have been covered by the agar. During the first two examinations it was easy to judge if the mycelium and the spores produced by this mycelium developed on the nail itself, and that is what I mean by invasion. This is not quite correct and it needs some explanation: by invasion I mean that mycelium or spores creep over the surface of the nail, and not that mycelium penetrates into the nail itself. Actually I do not know if that happened during my experiment: I did not look for it, not that I do not consider this phenomenon important, but simply because I had no time to do it properly. So, at the completion of the experiment, before covering the culture with collodion, the nails were simply removed and discarded.

The development on the surface of the nail was estimated by the presence of mycelium or spores on its surface. The importance of this development was very different not only from the first observation (after 5 days' growth) but from the second one (after 10 days' growth), and also from one nail to another. It will become apparent in the results that whereas the nails of the controls or of the animals that had been fed griseofulvin for a short period only were 100% or nearly so invaded by the dermatophyte, a good number of nails from animals fed griseofulvin for a sufficiently long time escaped invasion.

What will not be apparent in the tables is the importance of the invasion of the nails or the rapidity of their invasion by the dermatophytes. In a very general way, the presence of macroconidia for *Microsporum gypseum* and of microconidia for *Trichophyton mentagrophytes* indicated a heavy development of the fungus on the surface of the agar and of the nails. The presence of macro- or microconidia was different in the nails of the controls from what it was in the nails of the griseofulvin-fed animals. For instance, after 5 days' growth, the nails of 21 days griseofulvin-fed animals submitted to *M. gypseum* had 13 invaded nails out of 70 but only 3 had macroconidia, and after 10 days if 33 nails were invaded, 26 had macroconidia. On the contrary, nails of the controls after 5 days had 136 invasions on 140 nails and 96 macroconidia, whereas on the tenth day every one of the 140 nails was not only invaded but presented also a tremendous number of macroconidia. The same may be said of the nails exposed to the invasion by *T. mentagrophytes*: in the 21 days griseofulvin-fed animals, if after 5 days 10 nails were invaded out of 70, 3 only presented microconidia and after 10 days 49 were invaded and 36 had microconidia. On the contrary, the nails of the controls confronted with the same dermatophyte had, after 5 days, 100 nails invaded out of 143 and 68 with microconidia, whereas after ten days 142 nails out of 143 were invaded, all with microconidia. I should add that the number of macroconidia for *M. gypseum* and of microconidia for *T. mentagrophytes* was not exactly comparable in the nails of the experimental animals. In the first group the number of spores was usually reduced, and I accepted their presence as soon as I could see them, whatever their number. For the nails of the controls the growth was so heavy that the nail was completely altered in its outside morphology and looked like a little heap of sand.

In the results presented in the tables, I have taken account of the invasion, small as it may have been, on the two times of my observations after 5 days and 10 days; as soon as some mycelium developed on the surface of the nail I considered the nail invaded. That way of counting the invaded nails seemed the only possible one, but was actually in favour of invasion as against non-invasion.

Another fact that will not appear in the table is the development of the culture on the under surface of the slides. When the growth of the dermatophytes is not inhibited by the presence of some antibiotic, the growth is approximately the same on both surfaces: it is actually easy to observe that the development on the under surface is in agreement with the development on the upper surface. On the contrary, when we test nails of animals fed griseofulvin for a sufficiently long period, the presence of those nails on the upper surface of the slide inhibits the growth of the dermatophyte, but on the upper surface only: consequently the growth on the under surface is much faster. In the best cases it can be observed that the dermatophyte is developed on the entire surface of the underface of the slide, whereas on the upper surface it covers one-third or one-half only of the surface.

	No. of mice	No. of nails	No. of nails invaded after 10 days	% of invasion
Microsporum Gypseum				
Control	8	140	140	100%
Griseofulvin 15 days	4	66	66	100%
21 ,,	4	70	33	47.1%
28 ,,	4	71	44	62.9%
Griseofulvin 15 ,,	4	70	63	90%
+Azepine 21 ,,	4	71	53	74.6%
Baby mice of 28 ,,	3	51	49	96.1%
Trichophyton Mentagrophytes				
Control	8	143	142	99.3%
Griseofulvin 15 days	4	70	70	100%
21 ,,	4	70	49	70%

TABLE II.—NAILS INVADED AFTER 10 DAYS

Results

The results observed are reported in detail in Tables II and III. They may be summarised as follows:

Nails of mice not fed griseofulvin were invaded in 100% by *M. gypseum* and in 99.3% by *T. mentagrophytes* (series IX and X).

Nails of mice 15 days griseofulvin-fed were invaded in 100% either by *M. gypseum* or by *T. mentagrophytes* (series I and II).

Nails obtained from mice born from a mouse that received griseofulvin for 25 days were invaded in 96.1% by *M. gypseum* (series VIII).

Nails of mice fed 21 and 28 days griseofulvin were invaded, respectively in 47.7% and 62.1% by *M. gypseum* (series III and IV).

Nails of mice fed 21 days griseofulvin were invaded in 70% by *T. mentagrophytes* (series V).

Nails of mice fed for 15 or 21 days griseofulvin and azapetine were invaded in 90% and 74.6% respectively by *M. gypseum* (series VI and VII).

Microsporum Gypseum		No. of mice	No. of nails	No. of nails invaded after 5 days	% invasion after 5 days	% invasion after 10 days
	Control	8	140	136	97.1%	100%
Griseofulvin	15 days	4	66	62	93.9%	100%
	21 ,,	4	70	13	18.6%	47.1%
	28 ,,	4	71	22	31%	62.9%
Griseofulvin	15 ,,	4	70	18	25.7%	90%
+Azepine	21 ,,	4	71	29	40.8%	74.6%
Baby mice from mother 28 days griseofulvin		3	51	47	92.1%	96.1%
Trichophyton Mentagrophytes	Control	8	143	100	70%	99.3%
Griseofulvin	15 days	4	70	31	44.3%	100%
	21 ,,	4	70	10	14.2%	70%

TABLE III.—NAILS INVADED AFTER 5 DAYS

Table III indicates that when the nails come from an animal that has not received griseofulvin or received it for too short a period the number of nails invaded after 5 days is much greater (and sometimes as much as after 10 days or nearly so) than when the nails come from animals which have received sufficient griseofulvin. This is less evident for *T. mentagrophytes* which grows slower than *M. gypseum.*

		No. of mice	No. of nails on forepaws	No. of nails on forepaws invaded	No. of hindpaw nails	No. of hindpaw nails invaded
Microsporum Gypseum						
	Control	8	64	64 (100%)	76	76 (100%)
Griseofulvin	15 days	4	28	28 (100%)	38	38 (100%)
	21 ,,	4	31	11 (35.5%)	39	22 (56.4%)
	28 ,,	4	31	12 (38.7%)	40	32 (80%)
Griseofulvin	15 ,,	4	31	28 (90.3%)	39	35 (89.7%)
+Azepine	21 ,,	4	31	23 (76.1%)	40	30 (75%)
Young mice born to mother after 28 days		3	21	20 (95.2%)	30	29 (96.7%)
Trichophyton Mentagrophytes	Control	8	63	62 (98.4%)	80	80 (100%)
Griseofulvin	15 days	4	31	31 (100%)	39	39 (100%)
	21 ,,	4	31	14 (45.2%)	39	35 (89.7%)

TABLE IV.—INVASION OF THE NAILS OF THE HIND AND FOREPAWS

Every nail was observed individually for invasion: I could therefore say exactly which nail had been protected or invaded for each mouse. I am afraid that this would not get us very far. I have, however, made a comparison between the number of nails invaded for the fore paws and for the hind paws (Table IV). It is clear from this table that the nails from the hind paws were more invaded than those from the fore paws:

that is true except for nails from animals fed with griseofulvin and azapetine. If we omit this exception we could conclude that the nails of the fore paws contain more griseofulvin than those of the hind paws.

WHITE MICE	1	2	3	4	5
Total weight	14.5 gm.	25.4 gm.	24.5 gm.	21.7 gm.	16 gm.
Weight of nails on right hind paw	1.9 mg.	1.1 mg.	1.4 mg.	1.4 mg.	1.4 mg.
Weight of nails on left hind paw	1.2 mg.	1 mg.	1.4 mg.	1.4 mg.	1.4 mg.
Weight of nails on right front paw	0.9 mg.	0.6 mg.	0.82 mg.	0.8 mg.	0.8 mg.
Weight of nails on left front paw	0.6 mg.	0.6 mg.	0.8 mg.	0.8 mg.	0.8 mg.

TABLE V.—WEGHT OF THE NAILS OF HIND AND FOREPAWS

I thought it useful to compare the weight of the nails of the fore and hind paws. I did this for 5 mice which were not used for the above experiments but which were from the same batch and of the same average weight as the mice used in the experiments (see Table V); curiously enough the weight of the nails of the hind paws is nearly twice the weight of the nails of the fore paws.

DISCUSSION

The results obtained throughout this experimental work indicate that when mice have received 1.25 mg. griseofulvin daily by mouth for a period of at least 3 weeks some of their nails are protected against invasion by *Microsporum gypseum* or by *Trichophyton mentagrophytes in vitro*. The lowest percentage of nails invaded was 47.1%; or to express it differently, the highest percentage of nails resistant to the invasion was 52.9%. The percentage of resistance did not in this experiment increase when the time of administration of the drug was 4 weeks. That may be a variation in a group of animals. It would be interesting to feed mice with griseofulvin for longer periods comparable with those used in human therapy.

When a substance with vasodilating properties, like azapetine, was added to the griseofulvin feeds the results were no better. On the contrary, the number of nails protected against invasion *in vitro* by *M. gypseum* in animals fed for 21 days with griseofulvin and azapetine was actually lower than for the nails of animals given griseofulvin only. The reason for this difference could lie in the diuretic action of the azapetine, but this action has not been demonstrated in mice, and there was no increase in the elimination of griseofulvin in the urine. However, the nails of animals fed for 15 days are invaded in only 90% of the cases.

Griseofulvin does not seem to pass the placental barrier: the 3 mice born from a mother fed with griseofulvin for 25 days favour this conclusion since their nails were invaded in 96.1% by *M. gypseum*.

The nails protected against dermatophytes *in vitro* are more often the nails of the fore paws than the nails of the hind paws. The weight of the hind paw nails is twice that of the nails of the fore paws. It would be difficult to explain the higher protection

of the nails of the fore paws by the fact that the mice could lick their fore paws. Mice fed with griseofulvin for 15 days do not have nails resistant to dermatophytes. We should then have to accept the view that griseofulvin may accumulate in the nail from outside.

The number of animals used in these experiments was small: 47, and they produced 802 nails.*

I think that the experiment presented in this paper should be done on a larger scale, but whatever the results obtained in the future experiments I feel sure that, if not a complete confirmation of my work, some interesting facts will be found.

Microscopic examination of the stained slides has failed to show any abnormalities of the hyphal filaments. That is to say, the "curling effect" was not observed on the slide cultures whether the mice had been fed with griseofulvin or not.

	No. of nails	No. of nails invaded after 10 days	% of invasion	2 s' p	t.s p	Limits of invasion	
Microsporum Gypseum							
Control	140	140	100.00%	0			
Griseofulvin							
15 days	66	66	100.00	0			
21 days	70	33	47.14	24.92	9.78	37.36%	56.92%
28 days	71	44	61.97	23.57	9.51	52.46	71.48
Griseofulvin + Azepine							
15 days	70	63	90.00	9.00	5.88	84.12	95.88
21 days	71	53	74.65	18.92	8.53	66.12	83.18
Mice born from mother fed on griseo 25 days	51	49	96.08	3.77	3.80	92.28	99.88
Trichophyton Mentagrophytes							
Control	143	142	99.30	0.70	1.63	97.67	100.00
Griseofulvin							
15 days	70	70	100.00	0			
21 days	70	49	70.00	21.00	8.98	61.02	78.98

TABLE VI

I have submitted my results for mathematical analysis to Doctor Leopold Martin, Professor of Statistics at the Université Libre de Bruxelles. The results of his analysis will be published in the Review "Biometrie Praximetrie." They indicate (Table VI) that the difference between animals fed griseofulvin for 21 days have a statistical value when compared with the controls and with the animals fed for 15 days; that these results have no value when compared with the animals fed griseofulvin for 28 days; that the results for animals fed 15 days griseofulvin and azapetine have a statistical significance when compared with the controls and the animals fed 15 days griseofulvin alone; that the differences between animals fed 21 days griseofulvin and the animals fed 21 days griseofulvin and azapetine has a real statistical value; that the young mice born from a mother fed 25 days griseofulvin compares with the controls.

* The ideal number of nails should have been 846. The 44 missing nails were simply not present on the animals used.

SUMMARY

Some cases of *Trichophyton rubrum* infections of the nails and their treatment by griseofulvin are described. Of the 7 cases treated, 2 were completely cured after 5½ months and 8 months, and 5 others have been considerably improved. The drug tolerance was good. The dosage was usually 2 gm. a day for the first month, 1 gm. a day subsequently. An attempt was made to demonstrate that the nails of mice fed griseofulvin are not equally protected against the invasion by two dermatophytes, *Microsporum gypseum* and *T. mentagrophytes.* The mice which have been fed 1.25 mg. griseofulvin a day for 15 days react just the same as mice which have not received any griseofulvin. When griseofulvin was given for 21 days, 47.1% of the nails were invaded by *M. gypseum*—instead of 100% in the controls: in other words, 52.9% of the nails were protected. The addition of a vasodilating drug to griseofulvin does not seem to improve its assimilation by nails.

Acknowledgements

My thanks are due to the two Carmelite nuns and Miss Feyen who have helped greatly with the technical part of this work. I am also very grateful for the generous help given by Glaxo Laboratories, Imperial Chemical Industries, and Wolff (Antwerp) in furnishing the griseofulvin necessary for the clinical and experimental work. I am greatly indebted to my colleague Professor Martin for his help with statistics.

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DISCUSSION

DR. TICKNER: There are two questions I should like to ask Mr. Tomich. First, why is there any difference in the amount of fluorescence you get between one per cent ethanolic and pure aqueous solutions of griseofulvin? Has he tried chromatography at all to separate out griseofulvin from tissue extracts? Unfortunately, we at the Institute of Dermatology have no spectrofluorometer. We have an ordinary fluorometer and therefore we have to be rather more rigorous in extracting our griseofulvin. We have tried extracting from skin with the aid of chromatography on alumina. We have so far been unsuccessful as there is something in skin which fluoresces and has the same chromatographic characteristics as griseofulvin has on alumina. We are now trying silicic acid.

MR. TOMICH: Griseofulvin dissolves in water to the extent of about 10 micrograms per ml. We prefer to make up accurate standards by dissolving pure griseofulvin in pure alcohol and then diluting it down to one per cent alcohol. We use that as our standard. Therefore, to be exactly correct, we isolate griseofulvin and then dissolve it in the same solvent, which has no fluorescent activity at all. It gives about half the value which a solution of 0.05 micrograms griseofulvin will do.

The second question related to chromatography. We have done no chromatography at all at Greenford. All I can say about interfering fluorescent bodies in the skin is that the best thing you can do is to use a spectrofluorometer because you will not get that trouble.

DR. BROSSI (Basle): I have two questions for Mr. Tomich. First, were you able to isolate from tissue extracts metabolic products of griseofulvin, and have you been able to determine their structure?

Secondly, I believe that all compounds with the same chromophore system as contained in griseofulvin should show the same UV-Spectrum and as far as I know they probably will do the same in fluorescent light. So this method would not be specific to griseofulvin alone. I wonder what difference, for instance, you have noticed using your method comparing griseofulvin with isogriseofulvin?

MR. TOMICH: With regard to your question on the isolation of metabolites from animal tissue, the answer is yes. Rats were given oral doses of griseofulvin and the urine was collected and sent to our colleagues at Sefton Park. They have shown that animals remove the methyl group at the 6 position, leaving the corresponding hydroxy compound. We confirmed this also *in vitro*. Do you remember the graph showing the liver incubation experiment? If you incubate griseofulvin with liver slices and when all the griseofulvin has disappeared look at the supernatant liquid again, you will find exactly the same metabolite. I do not think we know any other.

The second question was on isogriseofulvin. We have not looked at that compound.

DR. CHATTAWAY: There are two points I should like to raise with Mr. Tomich. I was interested to hear that recovery of griseofulvin was low, particularly from skin. I should like to ask whether this was due to the interference of fat which he mentioned later, or whether the griseofulvin was in fact not extracted from the skin by the ether, the inference being that it might be more firmly bound, since we are interested in the specific uptake of griseofulvin into skin. If it is a question of the fat, do I take it that it was whole skin which was used? Would it not be possible to remove the adipose fat from skin and see what the recovery of griseofulvin was like then, because it is a point of some considerable interest to see if griseofulvin is more firmly bound in this tissue than it is in others?

Then he mentioned the varying ability to absorb griseofulvin from different parts of the alimentary canal. Can he give us any idea of the percentage absorption of a dose of griseofulvin? Also, can he tell us whether this varies from one species of animal to another, because some of the difficulties of treatment might be applicable in terms of differences in absorptive capacity?

MR. TOMICH: We believe that the poor recovery from skin is due to the fat content. We used whole skin. However, our colleague, Dr. Muggleton, has recently done some work taking guinea-pigs, dosing them orally, and then taking a blood sample, a whole skin sample, a skin sample laid out on a board and superficially scraped, and then a hair sample. The ratios of griseofulvin in those four tissues were: blood, one; whole skin, two; superficial layer, five; and the hair, twenty. That sounds very interesting and encouraging, but I know that if Dr. Muggleton were here he would agree with me that there is one possible snag in it: that is, although you clip the hair off from the skin you cannot get down to the roots and follicles, and because there is such a high concentration in the hair it is just possible that the outer layer appears to have more griseofulvin than it really does.

With regard to absorption from the alimentary tract, originally we thought griseofulvin was not absorbed at all from the alimentary tract because it has such an extremely low oral toxicity. As you know, you can dose mice with 50 grammes/kilogramme and they are not bothered a bit. Then we heard of Dr. Gentles' work and that proved that the stuff was absorbed. Our colleagues at Sefton Park have used radioactive griseofulvin and the results vary quite a bit. These experiments were done in rats, and they have had recoveries of radioactive chlorine—that does not mean griseofulvin at all—in the urine from these rats over twenty-four hours, varying between ten per cent and forty per cent. However, I think better light is thrown on your question by some experiments which Mr. Davis has been doing recently at Greenford. He has been giving rats single oral doses and then at intervals killing them off and assaying the whole of the alimentary tract content for griseofulvin. Also, groups of rats were allowed to pass faeces until no more griseofulvin came through. Usually with the old technique with ether we only got sixteen per cent in the faeces, but using acetone we are now getting sixty per cent quite regularly. At various levels between twenty and one hundred milligrams per kilogram we generally get about sixty per cent of the orally administered dose coming out as griseofulvin in the faeces.

THE CHAIRMAN: Mr. Tomich, when are we going to know whether it is griseofulvin in keratin or not? How much keratin from a human being would you want to find this out? Thirdly, what about nails? Do we know whether it is griseofulvin in nails? How many nails would you like to find out? A lot of us would send you them.

MR. TOMICH: I would say send as much as you can.

THE CHAIRMAN: I hoped you might have reduced it by now.

DR. CHILD: I should like to ask Dr. Riddell a question. I was interested in the sensitivity he was quoting for *Microsporum canis* assay, because it was much greater than I had realised from the work done by Dr. Muggleton. I wondered if the figure he was quoting was a final dilution figure and not the actual amount in the blood. I should like to ask him if he really can detect that low level in the serum.

DR. R. W. RIDDELL: That level was quoted from Blank and not our results. The way this was derived I imagine that degree of delicacy was due to the micro-methods he was using. Ours were about 0.3.

DR. CHILD: That is the sort of sensitivity I would have expected.

DR. WHITTLE: Following up the question about nails, I was wondering if, in view of what Vanbreuseghem has been saying on the extreme variability of the infection of nails, and the variability of response so that one gets the paradoxical effect of clearing up toe-nails and being left with finger-nails infected (which is quite the wrong way round to what you might call the ordinary experience), it would help us if we could get information on the factors involved. I was wondering whether it is a question of circulation or other factors such as hormones playing a part. Mr. Tomich, you say you want large quantities of nails. What do you mean by large quantities?

DR. CHILD: May I answer that question, Mr. Chairman? We have had quantities up to ½ gramme nail clippings from Dr. Everall and we have not been able to detect any griseofulvin, although they were from patients receiving the drug. However, that does not mean it was not there; we probably could not get it out.

At the same time we received scrapings from the soles of the feet of patients receiving griseofulvin, and I think in every case except one we failed to detect griseofulvin. In one patient we detected griseofulvin in the sole scrapings, and then it must have been a high concentration because we only had a matter of 10 to 15 milligrammes of sole scrapings.

THE CHAIRMAN: Is it true that you really want about 2 grammes of either of these materials?

DR. CHILD: We just want as much as we can get.

DR. CHATTAWAY: May I ask whether you have tried degrading the keratin at all, because if the griseofulvin is firmly bound it may affect the fungus simply when it attacks the keratin? If you degrade keratin to some extent can you recover more griseofulvin?

DR. CHILD: We have not tried degrading keratin, but we have tried macerating the hide of animals with barium sulphide and things of that nature. We have always found that if you add these other things you get so much irrelevant fluorescence that you cannot assay.

DR. BROSSI: I should like to discuss the paper of Professor Vanbreuseghem. I should like to ask him if I have understood him correctly. The nails were taken from animals which were receiving griseofulvin. What would happen if the extraction of the nails were done two, three or five days after suspension of treatment? I think his findings have been confirmed by German and French authors. The German authors have put a hair from griseofulvin-fed animals on agar blocks inoculated with dermatophytes and have shown that no development of the dermatophyte occurs. On the other hand, French workers have demonstrated that hairs of griseofulvin-fed animals are not invaded, are not penetrated. However, in both cases we do not know if these hairs have been extracted immediately or during the treatment. What would happen if the treatment were suspended some days before doing these assays? In other words, how long can griseofulvin be demonstrated in these tissues after suspension of the feeding of the substance?

PROFESSOR VANBREUSEGHEM: I cannot answer what I do not know. I think I exposed the problem to you. It would be interesting to suspend the administration of griseofulvin for a few days and see what happens.

There is one point I did not raise and I hoped you would do it for me: that is, what would happen if, instead of three or four weeks, I was giving griseofulvin for eight weeks or for a longer time? Would I still have the same differences between the controls and the animals receiving griseofulvin? I am not sure at all. It is something we shall have to repeat and extend this experiment.

DR. PEGUM: I do not think Professor Vanbreuseghem told us how long a mouse's nail takes to grow. I take it that is an important point, because presumably one would want to do it for the time it takes a mouse's nail to grow from start to finish so that the whole thing could be thoroughly permeated with griseofulvin from one end to the other.

PROFESSOR VANBREUSEGHEM: You have probably observed that I am not a "mouse-ologist." What I did was to take mice in the same state of good health and weight, and to see how it went. But I do not know the answer to your question. The results were produced by the difference in weight between the fore and the hind paws.

THE CHAIRMAN: Does anyone know how long a mouse's nail takes to grow? Not even a scientist?

DR. BARLOW: Can I ask Mr. Tomich how long griseofulvin is stable in animal tissue? The point is that the big toe-nail takes at least twelve months to grow out, and therefore with the distal part the griseofulvin would have had to be given roughly twelve months ago to be effective now.

MR. TOMICH: I do not know the answer to that question. All I can tell you is that there are very few chemical substances more stable. You can sublime griseofulvin and it is still griseofulvin. If it stays stable under those conditions it is possibly stable in the tissue. That is the best I can do.

THE CHAIRMAN: We will give you a terminal bit of nail to estimate.

MR. TOMICH: All right, in twelve months' time.

ACTION OF GRISEOFULVIN ON METABOLISM OF T. RUBRUM IN VITRO

A. Tickner

Although many, perhaps most, of the useful drugs introduced into medicine have arrived empirically, it is always intellectually satisfying to know how they work. Moreover, information acquired during the search for insight into their action may give rise to fuller understanding of some aspects of biochemistry or physiology, or it may suggest fruitful modifications of existing drugs or even useful indications of new ones.

For these reasons, my colleagues P. D. Mier and M. McCabe and myself have started some investigations into the biochemical action of griseofulvin upon Trichophyton rubrum. We have so far investigated the action of griseofulvin upon the respiration, proteolytic activity, phosphatase and transaminase of T. rubrum and on some aspects of phosphorus metabolism of this organism. We here hesitantly, and perhaps prematurely, report on our findings.

Materials and Methods

Cultures were obtained in the first instance from the Department of Mycology at the Institute of Dermatology. For the experiments on proteolysis, phosphatase and transaminase, the organism was grown on Sabouraud's broth for 14 days at 30°C. At the end of this time, the mycelium was harvested and freeze dried. The dry mycelium was ground and extracted with 0.2 M. phosphate buffer for 10 minutes at room temperature. The solution was filtered and dialysed against distilled water at 4°C.

Proteolysis

For measurement of proteolysis, 1 ml. portions were added to 10 ml. of 1% casein solution in phosphate buffer at pH 7. The mixture was incubated at 37°C and 1 ml. samples were taken at specified times. To these samples were added 3 ml. of 5% trichloroacetic acid in centrifuge tubes. The tubes were spun at 3,000 r.p.m. for 15 minutes and the optical density of the supernatant was measured at 280 mμ. In this and all other experiments concerning griseofulvin, 6 portions were assayed for metabolic activity, and to 3 of these portions solid griseofulvin was added to give a saturated solution.

Phosphatase

This activity was measured at pH 5 or 10 in citrate or glycine buffer respectively. The splitting of disodium phenyl phosphate was followed by the measurement of inorganic phosphate by the method of Berenblum and Chain as modified by Ernster (1950).

Transaminases

Glutamic oxalacetic and glutamic pyruvic transaminases were measured by the method of Wroblewski and Cabaud (1957).

Respiration

Cultures of T. rubrum were grown for a week on Sabouraud's broth. The mat was washed in sterile 0.9% sodium chloride solution and about 10 mg. of the mat was cut out using a sterile cork borer. The disc of fungus was added to a sterilised cup of a

Cruickshank differential microrespirometer. Then 0.02 M. glucose in phosphate buffer at pH 7.0 was added to each side. Three respirometers were used as controls while to three others solid griseofulvin was added. The oxygen uptake was measured at intervals.

Acid Soluble Phosphates

Eight flasks of Sabouraud's broth containing 20 microcuries of P per ml. were inoculated with T. rubrum and growth was allowed to proceed for 5 days at 30°C. At the end of this time griseofulvin sufficient to saturate the medium was added to half of the flasks. At the end of 1, 3, 5, and 7 days further growth respectively, a control and a griseofulvin-treated flask were treated in the following manner. The mat was collected and washed and dispersed in 2% trichloroacetic acid such that the final dispersion contained 50 mg. fungus per ml. The dispersate was centrifuged and enough of the supernatant to contain about 20,000 counts per minute as measured at 1 cm. in air from an EW 3H counter, was spotted on to the corner of a large sheet of paper (Whatman N° 541 for chromatography). The paper was then subjected to two-way descending chromatography followed by radio-autography using the method of Brooks et al. (1959).

Results

It was discovered that T. rubrum possesses active transaminases and an alkaline but not an acid phosphatase, which do not appear to have been previously reported. None of these enzymes nor the proteolytic enzyme of T. rubrum is affected by griseofulvin. Respiration in the presence of glucose was depressed slightly but significantly (to 89% of control values) in the first 24 hours, but was thereafter unaffected.

Many (15-20) acid soluble phosphate spots could be observed following chromatography and radio-autography. After one day's treatment with griseofulvin no change in the pattern of these was noted, but after three days two new spots appeared having Rf relative to inorganic phosphate of 0.19 and 0.26 in the alkaline solvent, and 0.06 and 0.10 in the acid solvent respectively. By the fifth day these spots had gone, and the pattern then and on the seventh day was essentially similar to the controls.

Discussion

We have found a slight inhibition of respiration of T. rubrum by griseofulvin, from which, however, the fungus escapes after 24 hours. This same slight depression of respiration was also observed by Roth, Sallman and Blank (1959), although since these author's experiments lasted only 60 minutes, the subsequent lack of inhibition was not seen. It is tempting to relate the subsequent temporary aberration in phosphorus metabolism to this temporary inhibition of respiration but such a link is mere speculation.

As the nature of these transiently appearing phosphorus compounds is unknown it is idle to make any conjectures as to their significance. It is sufficient to point out that a temporary disturbance of metabolism of nucleotides could have consequences continuing to act long after the disappearance of these nucleotides from the acid soluble fraction.

In conclusion I cannot over-emphasise the part played in these investigations by my two young colleagues. Although it has been my lot to talk about this work, theirs has been the task of adapting and perfecting the techniques which have made it possible.

SUMMARY

Griseofulvin has no effect in vitro upon the glutamic oxalacetic transaminase, glutamic pyruvic transaminase, the alkaline phosphatase or the protease of T. rubrum.

It does cause a slight transitory decrease of respiration and also the transient appearance of abnormal phosphorus-containing compounds in the acid soluble fraction.

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INVESTIGATIONS WITH RADIOACTIVE GRISEOFULVIN

A. Scott

Once the efficacy of griseofulvin had been established in the treatment of clinical and experimental infections with the superficial dermatophytes, it became of interest to trace its localization and behaviour in the skin. It was hoped thereby to gain some understanding of the means by which this material was able to alter the usual pattern of fungal infection. It had already been established by spectrofluorophotometric analyses that griseofulvin showed a preferential concentration in skin after its oral administration. Our present purposes then were

(1) to demonstrate its localization in skin, together with any particular structures in which it tended to accumulate;

(2) to determine the optimum time for this concentration;

(3) to trace any pattern of behaviour illustrated by griseofulvin in the skin.

Methods

The griseofulvin molecule lends itself to the incorporation of a radioactive ion during its preparation by virtue of the presence of a chloride ion in one of its rings. If potassium chloride Cl(36) (beta negative decay; half-life of 4.5 x 10^5 years) be added to the fermentation mixture, approximately 10% of the chloride will be incorporated into the griseofulvin produced. By this method material with a specific activity of 6.7 microcuries per gram was obtained for experimental tracer work.

Two sets of experiments were undertaken. In the first, normal rats were fed by tube with 100 mg./Kg. of griseofulvin-Cl(36) on three successive days. The animals were sacrificed at 4, 7, and 24 hours after the last dose had been given. Portions of the skin were submitted for spectrofluorometric and radio-assay, and for autoradiographic studies. As controls for this group, a normal untreated rat was examined, as well as three rats which were similarly tested after the administration of DL-cystine-S(35) (specific activity of 155 microcuries per gram) on three successive days.

In the second study, twelve patients with cutaneous fungal infections due to Microsporon audouini or Trichophyton rubrum were examined. Ten milligrams of griseofulvin-Cl(36) contained in a conventional liquid paraffin-water gel were applied to each tinea lesion, and covered with a cellophane patch. Biopsies were taken when the material had been in contact with the skin for 4, 8, and 24 hours. Geiger counts were done over the test sites after application of the radioactive griseofulvin and again immediately prior to biopsy after cleansing the area with water and acetone.

Autoradiographs were made from 10 micra sections of the biopsies from human skin and from rat skin. These were exposed to Kodak A.R. 10 and A.R. 50 photographic emulsion for periods of six weeks at -4°C. Routine development and fixation were carried out. Some of the serial sections were stained by the PAS method to demonstrate the position of the fungi.

Controls in the second study consisted of biopsies from normal human skin similarly treated.

Results

Examination of the autoradiographs in both studies directed our attention principally to the hair follicles and the stratum corneum which were almost the sole sites of concentration of radioactivity (Fig. 1). Since the pattern of autoradiograph produced in the rats treated with DL-cystine-S(35) served as a basis of comparison for the other results we shall consider these first. Our findings were identical with those reported by

Bern and Belanger (1957) working with sulphur(35) both as sulphate and incorporated into DL-cystine. Sulphur(35) was concentrated in the suprabulbar, keratogenous zone of the hair follicle. All the follicles with the exception of those in the telogen phase showed a dense accumulation of radioactivity. Furthermore the sulphur was distributed through most of the hair shaft, suggesting that the radioactive material was being incorporated in the keratin. The epidermis also contained radioactive grains throughout all its layers, and especially in the stratum granulosum.

Study of the autoradiographs made from skin treated with orally administered griseofulvin-Cl(36) demonstrated that the chloride(36), like sulphur(35) had accumulated in the suprabulbar zone of the hair follicle (Fig. 2). Unlike the sulphur(35), however, it was present only in those follicles which were in the actively growing anagen phase. The number of grains was more scant than in the cystine-treated group, nor did they occur diffusely through the hair shaft. Rather they remained confined in bands, apparently corresponding to the separated times of daily administration. Some radioactivity was also noted in the stratum corneum, usually around the orifices of the hair follicles. There was no evidence of radioactivity in the remainder of the epidermis.

In the second series of autoradiographs from human skin treated with local griseofulvin there was almost no absorption and localization in normal skin, but in biopsies taken from tinea lesions the radioactivity was readily demonstrable in the infected stratum corneum as well as adjacent infected hair follicles. The grains in the epidermis extended as far as the stratum granulosum, and particularly large clumps of grains were identified in positions corresponding to the sites of quantities of PAS positive fungal hyphae (Fig. 3). In the hair follicles radioactive material was located in the keratogenous zone and in the lowermost part of the hair shaft only. This latter finding was most likely due to the relatively short span of time of contact with the griseofulvin compared with the time necessary for hair shaft growth.

TABLE I

COMPARISON OF ASSAY RESULTS OF GRISEOFULVIN-CL(36)

	GRISEOFULVIN-CL(36) TREATED RATS	
TIME OF SACRIFICE AFTER LAST DOSE	4 Hours	7 Hours
TOTAL DOSE OF RADIOACTIVITY (μc/100G.rat)	0.2	0.2
RADIOACTIVITY OF SKIN (% of dose)	0.004	0.02
GRISEOFULVIN ASSAY SPECTROFLUOROMETRY (% of dose)	0.023	0.027
RADIOASSAY * (% of dose)	0.004	0.021
EXPECTED CONTENT (based on even tissue distribution — % of dose)		0.0027

* assuming all Cl(36) remains in griseofulvin.

Both spectrofluorophotometric and radio-assays of rat skins showed that 10% of the administered dose of griseofulvin-Cl(36) was concentrated in skin by the end of 7 hours. This close parallel of results indicates that during the entire three day sojourn of griseofulvin in skin studied, the griseofulvin molecule maintained a relative integrity. There is a discrepancy between the two results obtained at four hours which is most likely explained by the technical difficulties in the spectrofluorometric method. If one considers the radio-assay results alone there appears to be a gradual "build-up" in

concentration over the first seven hours after administration. The results at both time intervals give higher values than one would expect from an even tissue distribution of griseofulvin, in which approximately 1% would be expected to be localized in skin.

TABLE II

THE CONCENTRATION OF GRISEOFULVIN-CL(36) IN HUMAN SKIN

TEST AREA OF SKIN	NO. OF CASES	RADIOACTIVITY (COUNTS EXPRESSED AS % INITIAL VALUE)		
		4 Hours	8 Hours	24 Hours
INFECTED	2	2	1	1
NORMAL	12	2	9 — 18 (12%)*	12 — 22 (19%)*

* Mean values in brackets.

Geiger countings taken of normal human skin immediately prior to biopsy showed only 1 - 2% concentration in the skin at 8 hours, while readings taken over similarly treated tinea lesions revealed concentrations from 9 to 18%, indicating a much higher ability of this skin to absorb and retain the griseofulvin-Cl(36) at 8 hours and also at 24 hours. There was no difference in the readings obtained in the two groups at 4 hours.

Discussion

The close similarity in the distribution of griseofulvin-Cl(36) and DL-cystine-S(35) in the hair follicles and stratum corneum of the rat suggests that the former is also taken up during keratin production. The work of Bern (1957) and of Ryder (1957) with radioactive glucose-C(14) and anions such as phosphate-P(32) not directly concerned with keratin formation showed that these accumulated in the hair bulb, while sulphur(35) and, in this case, griseofulvin-Cl(36) entered the hair suprabulbar zone directly, thence into the hair shaft. Similarly, radioactive glucose and anions such as phosphate and chloride alone were localized in the basal layer of the epidermis and not in the stratum corneum, while sulphur (35) had a denser concentration in the stratum corneum and stratum granulosum, where the most active conversion occurred to soft keratin. From the same observations supplemented by the compared results of the spectrofluorometric and radio-assay of griseofulvin, one may conclude that the griseofulvin-Cl(36) molecule remained relatively intact during its sojourn in the skin. Chloride(36) by itself behaves as do the other anions.

Despite its association with keratinized tissue it was not felt that griseofulvin was intimately concerned with the keratinization process. Its distribution was limited to the actively growing phase in the normal skin, its localization was band-like in hair keratin corresponding to its interrupted administration; it did not appear in quantity in the uninvolved soft keratin of the stratum corneum, although it did concentrate in infected stratum corneum primarily around deposits of fungal hyphae. All these findings are in contrast with those observations made with DL-cystine-S(35) which is closely involved in keratin formation. In our own work with topically applied sulphur(35) as sulphate, reported elsewhere, we failed to find any concentration of radioactivity around sites of infection in the stratum corneum, although there was accumulation in the stratum granulosum and epidermal basal layer presumably because of active "parakeratinization". This fact would also contradict any suggestion that griseofulvin was behaving as an intimate part of the keratin molecule itself. It may be recognized, however, that some part of keratin has a particular holding action on griseofulvin, since

FIG. 1

× 65. This autoradiograph illustrates the deposition of radioactivity around hair follicles and within hair shafts of a rat fed on 3 successive days with griseofulvin—C1(36). Particularly evident on the right side of the photograph are the alternating bands of radioactive deposits and clear zones.

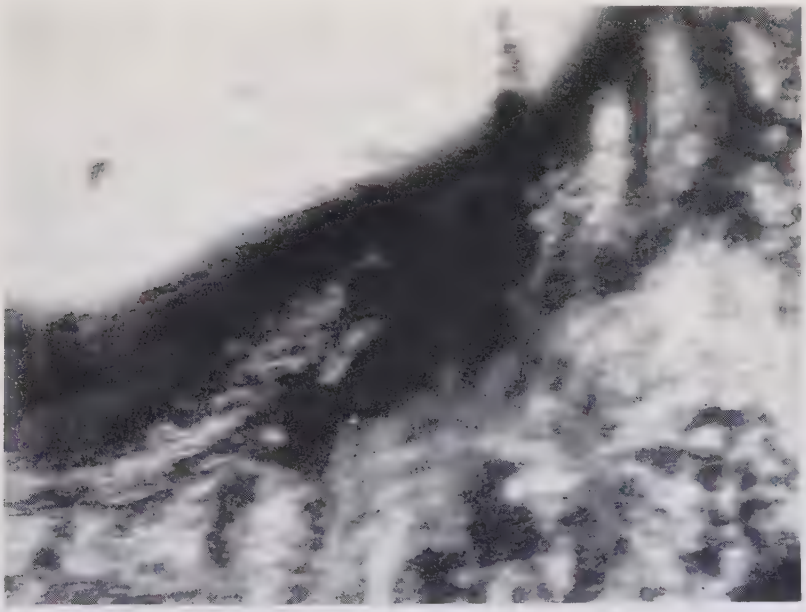

FIG. 2

× 350. Autoradiograph of a section of rat skin showing a dense deposit of radioactivity in the hair follicle and extending up the shaft. Some is also present in the stratum corneum adjacent to the follicular orifice.

FIG. 3 (a) x 970. A section of human skin stained with PAS to demonstrate fungal hyphae in the stratum corneum.

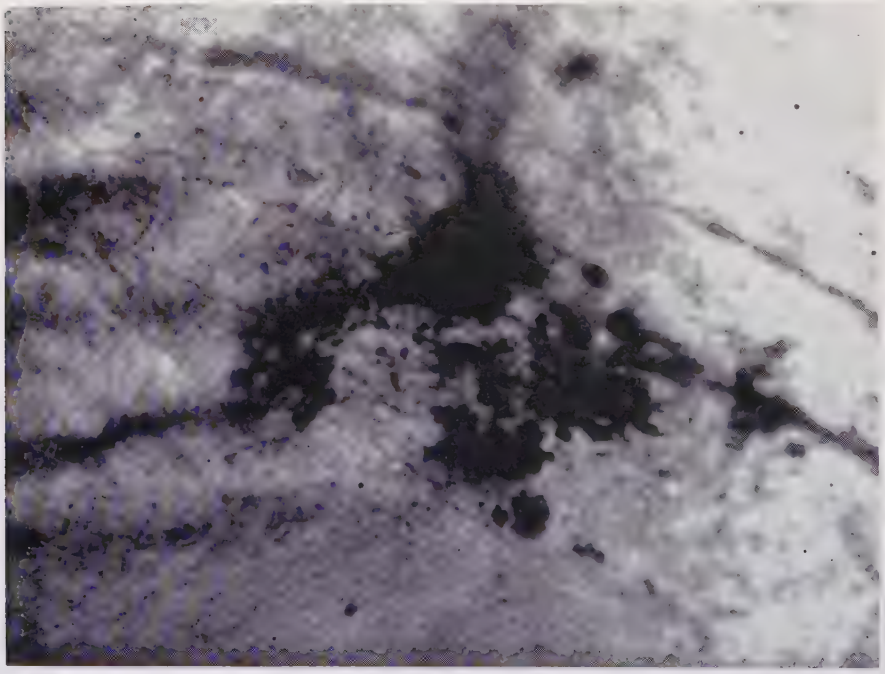

FIG. 3 (b) — The corresponding autoradiograph showing a dense cluster of radioactive granules at the site of fungal hyphae.

from our assays and Geiger readings a steady increase in concentration could be demonstrated in the skin, whereas the quantity of a material having no preferential localization would tend to vary with that circulating in the blood stream, and reach its peak level in 4 hours, thereafter decreasing as it was removed from circulation by the liver and kidney. No such parallel behaviour was observed here, indicating that the griseofulvin was specifically retained.

The relative immobility of griseofulvin-Cl(36) in the keratin precludes an intracellular function, but rather suggests either an adsorption onto certain free radicals of keratin or a fixation in the interstices of the keratin lattice. Our observation of the scant accumulation of griseofulvin in normal keratin compared with the much greater concentration of this material in infected keratin would make it appear that adequately formed keratin is less capable of holding it, and would be compatible with either theory. The interesting fact that the radioactive material tended to collect most densely in the sites of fungal hyphae opens one further possibility, namely, that the fungus itself also possesses a chemical structure which attracts the griseofulvin either by its very makeup or through its alteration of the environment.

SUMMARY

Two series of experiments have been carried out with radioactive griseofulvin-Cl(36). In the first study normal adult rats were fed the material by tube on three successive days before sacrifice. Skin from the animals was submitted to spectrofluorometric and radio-assays for griseofulvin and for its radioactive form, while samples were also used for autoradiography. As controls for the group normal, untreated rats were examined as well as rats fed with DL-cystine-S(35).

Employing the observations made in the cystine-treated group as a basis of comparison it was found that like the former, radioactivity in the test set accumulated in the suprabulbar zone of actively growing hair follicles and to a lesser extent in the stratum corneum adjacent to the orifices of the follicles. In contrast to the radioactive sulphur, the Cl(36) was deposited in bands in the hair shaft corresponding to the separate points of administration. A close parallel was noted in the results of the two types of assay, suggesting that the griseofulvin molecule remained intact during the time spent in the skin.

In the second study the radioactive griseofulvin was applied topically to lesions of tinea corporis, controls being served by similar applications to normal human skin. Geiger readings done during the time of exposure showed a higher rate of absorption in the diseased skin than in the normal (18% in contrast to 2%) while autoradiographs made from biopsies demonstrated penetration of the radioactive granules into the stratum corneum as far as the stratum granulosum, with dense deposition around the sites of fungal hyphae. The distribution in infected hair follicles was similar to that seen in the rat studies.

The two studies demonstrated the maintained integrity of the griseofulvin molecule, its specific retention in skin, particularly the keratin portion, and suggested a particular adsorption onto free radicals of keratin or fixation in the interstices of keratin.

ACKNOWLEDGEMENTS

I would like to thank the Glaxo Laboratories Ltd., Greenford, Middlesex, who not only supplied the radioactive griseofulvin, but also carried out the spectrofluorometric assays. Mr. Crocker, of the Pathology Department, St. Bartholomew's Hospital photographed the autoradiographs provided.

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DISCUSSION

DR. D. I. WILLIAMS: I must say how much I enjoyed the paper of Dr. Scott. It represents a great deal of well planned and thoughtful work. I hate to hark back to the clefts between the toes, about which I have an obsession. We know that various substances are well absorbed into the skin by the hair follicles. Since the areas which seem most difficult to free from fungus infections are those with no hair follicles, that is to say the palms, soles and clefts, it is tempting to believe that poor absorption may play at least a part in this failure. Clearly there must be other, perhaps more important factors. I would be interested to learn whether her experiments on absorption were carried out in these as well as in other areas.

DR. SCOTT: I should like to say that of the autoradiographs we showed, the second two from the last both came from a patient who had *Trichophyton rubrum* infection.

DR. JACKSON: I should like to ask Dr. Tickner in what medium his Trichophyton rubrum was during exposure to griseofulvin. Have you tried growing the organism in the presence of concentrations of griseofulvin which produced some degree of inhibition, and then exposing the harvested mould to labelled substrates, to see if growth in the presence of the drug has affected the way in which the substrates are metabolised?

DR. TICKNER: It was growing on glucose in phosphate buffer.

THE CHAIRMAN: Dr. Chattaway, can we invite you to comment on this?

DR. CHATTAWAY: I do not know that I have a lot to say, but I wonder whether Dr. Tickner has thought in terms of the original suggestion which was made, that the griseofulvin affects fungi with chitinous cell walls, and whether rather than looking at intracellular enzymes one might be able to detect changes in the cell wall when the organism is growing in the presence of griseofulvin.

I am very interested in Dr. Scott's observations, particularly that there is a greater uptake of griseofulvin in the infected skin than in the normal skin. Since it was a rubrum infection of the palm, are we to assume there was no inflammation there, or is this greater permeability explicable in terms of some inflammatory response of the tissue which may enable griseofulvin from blood to pass more readily from the site? It was obviously an observation of very considerable importance.

DR. JARRETT: I should like to support the last speaker. I should have thought that the increased uptake of radioactive griseofulvin in the infected skin was more likely to be related to the pathology of the keratin; perhaps the keratin is being produced more rapidly. That would account for the greater uptake of radioactive griseofulvin.

DR. SHAPERO: Dr. Tickner observed that griseofulvin caused a temporary depression of respiration of the organism. Can he suggest how he got this transitory depression? Is it a fact that the organism has used all the griseofulvin or changed it metabolically?

DR. TICKNER: As I understand it the question is whether the temporary depression was due to metabolic change in the fungus or because of the using up of all of the griseofulvin. It was not the latter since solid girseofulvin was always present. Further than that I cannot say.

DR. SHAPERO: You think it might be connected with some of the intracellular enzymes?

DR. TICKNER: One assumes it must be.

Incidentally, while I am on my feet, in reply to Dr. Chattaway, I think his suggestion is absolutely right. That is really why we switched the phosphate compounds in the hope of finding some abnormality of what we thought would be the precursors of the cell wall. If he has any suggestions as to how to break down the cell wall and investigate it, we shall be delighted to hear them. That is not meant in any way to complicate matters.

DR. WHITTLE: Do we really know that the keratin is formed more rapidly in these cases of rubrum infection?

THE CHAIRMAN: Who is prepared to answer that?

DR. WHITTLE: That is apropos of Dr. Chattaway's remark. I think it is a most helpful and stimulating remark and I wonder if there are facts bearing it out.

DR. CHATTAWAY: I do not think I made the remark about keratin being formed more rapidly.

DR. JARRETT: I made the remark regarding an increased rate of keratin production. Although the exact rate of keratin formation in human epidermis cannot be as yet accurately measured, I think it is safe to assume that when a thickened parakeratotic keratin is produced it can be taken as indirect evidence of an increased rate of keratin production. In fungus infections of the skin, changes in keratinization occur and these are sometimes parakeratotic. It is therefore quite likely that as far as our present knowledge goes that in these infections there is an increased rate of keratin production.

THE CHAIRMAN: Can Professor Vanbreuseghem give us any comments?

PROFESSOR VANBREUSEGHEM: I have mentioned blood changes in my paper. Actually, during the first month of administration of griseofulvin I have not observed any change, but there was some monocytosis in the third month. It was not very important except for one patient. After a single dose of 4 grams of griseofulvin this patient developed in her blood leukocytes with basophilic granulations at a rate which is usually considered as a manifestation of toxicity. When we stopped the griseofulvin and gave the patient some liver extract, the proportion of those leukocytes dropped to normal. So my conclusion is that there is some toxic effect, but it is not very important; only in exceptional cases is it necessary to stop the griseofulvin administration.

DR. AUSTWICK: May I suggest that there is one point which Dr. Brian brought out and which I fear has been overlooked? We are here today talking about a substance affecting a very small area of a particular fungal hypha. It is extremely small; I should say it is not more than 2 sq. on the end of a hypha. It does seem from Dr. Brian's work that it is only that area which is in fact protected by griseofulvin. Therefore, if you are investigating the effect of griseofulvin on the cell wall or investigating the metabolism of the fungus with which you are dealing — and that applies to whatever fungus it is — it is surely that area which you must concentrate on, which you must try and analyse. We know so very little about this hypha that it is a thing which I am quite sure, from the fundamental point of view, would bear a lot more investigation using a substance such as griseofulvin which we know has that effect. After all, due to our activities there are millions of fungal hyphae curling up their toes, as it were, all over the world because of this singularly peculiar effect on the growing point of the hyphae. It is no good using the hyphal wall behind the growing tip, you have to use just this very, very small area. We have to find out a lot more about it before we really know what we are doing.

THE CHAIRMAN: Thank you, Dr. Austwick, for bringing us down to earth again. May I ask you as a botanist how frequently this same phenomenon is observed in the absence of antibiotic action? I am thinking in terms of hyphal swellings, the sort of thing one sees in dysgonic dermatophyte cultures. Is this of the same nature?

DR. AUSTWICK: We have very little information on that. Dr. Robertson did some extremely interesting work to this extent on fusarium. I think Dr. Brian probably knows more about it than I do. He found that various treatments, such as water for just a few seconds, would cause hyphal swelling. When you see a hyphal swelling you do not realise that in fact this is a result of some influence — perhaps for a very, very short time — on the hyphal tip. I think that when you see a colony affected by griseofulvin you must think of it in terms of time and not just what you see. I do not

know whether Dr. Brian would confirm that, but there is no doubt that hyphal tips are extremely delicate things and they are the most important part of the hypha.

DR. BRIAN: May I just say a word or two about that? If you take a time-lapse photograph of the growth of a fungal hypha, normally you get the impression of growth taking place at the tip, as Dr. Austwick says. You see also that somehow or other the change in plasticity which must occur to allow that extension is controlled and oriented. In the presence of griseofulvin, the time-lapse photographs look very much as though the change in plasticity of the hyphal tip is disorganised. Hence you get these distorted forms because growth proceeds in abnormal directions, and so forth.

The work which has been mentioned really involved a change in tenacity of the suspending medium on which the fungus was growing and you get similar abnormal morphological forms. I think the point is that the two things are similar in so far as by changing the forces involved in extension and plasticity at the end you naturally get a similar expression. However, it does not lead you very much closer to understanding how it is that griseofulvin uses these changes.

THE CHAIRMAN: Can you help us, Dr. Chattaway?

DR. CHATTAWAY: No, Sir, I should like to know the answer myself.

THE CHAIRMAN: Dr. Gentles, have you any comments on these chemical aspects?

DR. GENTLES: No, Sir, I do not think I have any comments to make on this.

DR. OBAID: All the papers seem to be concentrated on the parenteral and oral administration of griseofulvin. No one, I think, commented on the local injection of griseofulvin into the skin or under the skin to see whether the effect would apply in that situation; the prolonged administration and the side effects of griseofulvin, in addition to the cost, is a great drawback for doctor and patient alike.

THE CHAIRMAN: What is the effect of injecting griseofulvin in the treatment of ringworm infections? Has anyone experienced this?

DR. FREY: I can only talk about the effect of locally applied griseofulvin on guinea-pigs with trichophytosis. We obtained the same curative effect as when we gave the substance by mouth. We were surprised by the fact that we saw the substance did penetrate to the Adamson fringe, i.e. to the keratogenous zone even when locally applied. We obtained the same stopping of the growth of the hyphae so that when the hair grew the distance between the keratogenous zone and the hyphae became longer. So we concluded that penetration of the drug in the hair by local application took place.

THE CHAIRMAN: With experimental infections I think I am right in saying that locally applied griseofulvin on the fourth day can be efficacious, but I am not familiar with the success of hair infections with griseofulvin applied locally. Can you help us on that, Dr. Gentles?

DR. GENTLES: There are probably some people here who can give more information on this. Perhaps Dr. Martin has some information on the local application of griseofulvin.

DR. MARTIN: As I said yesterday, there is some effect. It seems very variable, and in my experience it has never approached the best effect which you can get by oral administration. From what I have gathered in treating clinical infection in man, the effect is much less spectacular.

THE CHAIRMAN: We must bring ourselves back once again as we have rather drifted away from chemistry and got to the clinical field again. We would like to hear any final comments on these biochemical papers by Dr. Tickner and Dr. Scott before I ask Dr. Brian Russell to summarise.

QUESTIONER: Can anyone explain to me why it is that only the griseofulvin is localised with the inflammation and not the other radioactive substances? I may have missed a point in the paper, but I could not see why it was only the griseofulvin which was localised in the area of ringworm infection.

DR. SCOTT: We administered griseofulvin chloride and traced the radioactivity of

the chloride to the areas of infection by correlating the spectrofluorometric and radioactivity assays. We concluded they were one and the same.

QUESTIONER: That was not really the point of my question. I accept it was griseofulvin, but why was it localised when the other substances were not?

DR. SCOTT: The other substances?

QUESTIONER: The radioactive cystine was not localised round the area of infection?

DR. SCOTT: No. In the present series of experiments, DL-cystine-S^{35} served as control for the rats given oral griseofulvin — Cl^{36}. In other tests with topical $sulphur^{35}$ (Scott, A. & Kalz, F. Brit. J. Derm. 1956. 68: 52) we found no tendency for $sulphur^{35}$ to concentrate around areas of fungal infection. No experiments with $sulphur^{35}$ were done in this study to compare with the second group of biopsies done on human fungal lesions, but I think one may confidently compare the results of the topical griseofulvin — Cl^{36} behaviour with the above-mentioned $sulphur^{35}$ patterns.

DR. JACKSON: Can anyone say whether there is a marked pH effect on the activity of griseofulvin? If you measure its activity *in vitro,* is it strikingly sensitive to pH changes? Secondly, is it sensitive to alterations in salt concentration in the medium? Thirdly, someone suggested something about cytochrome systems. I wonder if anyone can tell us what cytochromes or other respiratory systems *Trichophyton rubrum* uses? I think myself that the respiratory effect is likely to be a secondary one. I would have thought that the idea that griseofulvin blocks pathways for the synthesis of cell wall materials was a better one for following up.

DR. WHITTLE: It would be rather useful to have information on the parallelism between these club changes which I think are concerned with the biochemistry of the organism, these distorted changes which you see in association with griseofulvin in the keratin in regard to the fungus, and also in healing lesions. I just wondered if you may not get changes occurring in lesions which are healing in the fungus somewhat similar to what you see with griseofulvin. I rather suspect that is happening although I have no proof of it. I suspect you get these distorted forms, which Dr. Austwick and others have mentioned, occurring in natural conditions without griseofulvin.

THE CHAIRMAN: Have we a respiratory enzymologist here to answer the first of these questions?

Could the fungal morphologists comment on Dr. Whittle's question?

DR. AUSTWICK: I do not know that I can actually confirm or really say that Dr. Whittle's observations have been borne out. However, there are certain changes you can detect, for instance in *Trichophyton verrucosum* infection in cattle when it is nearing its end, which you can really associate with the termination of the infection. The one with which I am really familiar is the fact that the arthro-spores instead of being round, which they normally are in the infected hair sheath and in the infected hair, fail to assume this round shape. They hardly assume the shape of spores at all; they are just simply hyphal segments which are flat-ended and rectangular or square. They are also slightly refractive.

As far as the hyphae are concerned, I think you are probably right in saying there are changes, but I am not familiar with the changes which griseofulvin causes in the actual hair itself. I have seen Dr. Gentles' slides but that is all, so I would not like to say that that is, in fact, comparable. For instance, we do not know why these infections terminate. We only assume that the animal develops immunity but we do not know why this happens at all. There is no doubt that the fungus stops growing down the hair.

DR. C. H. WHITTLE: This is outside the hair ones which I mentioned yesterday, definitely outside the two or three cases we saw. It is not in the hair at all.

DR. BRIAN: May I answer a question which was raised a moment ago? In my experience the activity of griseofulvin *in vitro* is not affected by pH in the range 3.5-7.0, nor is it affected by alterations in salt concentrations.

THE CHAIRMAN: Dr. Jackson, would you like to come back on that?

Dr. Jackson: No, I was just asking for information. One thing which occurs to me about Dr. Tickner's curve is that, if this experiment was done with fungus growing and the mass of the fungus was increasing during the course of the experiment, if that top curve is an indication of the oxygen uptake during the time, does it imply that in fact the uptake of oxygen per unit mass of fungus in the controls is decreasing over the whole of this period? You said the curve is effectively constant. I am not sure what the points on the curve were. If you measure the uptake over time intervals from time to time during the course of eight days, and they are always the same and the mass of fungus has increased, then the rate of the uptake of the fungus is falling in the controls.

Dr. Tickner: Experiments show the rate is constant per unit mass.

Dr. Brossi: This concerns the place of action of griseofulvin in the hyphae invading the hair. We saw in our control animals that healing follows the same pattern as we see under treatment with griseofulvin. We have also seen that the growing down of the hyphae is stopped when immunity arrives. This took place later than what happened when we were feeding griseofulvin. Also, we saw in the control animals that hyphae do not grow down, but are stopped and are growing up into the hair. There may be another substance acting in the same way as griseofulvin does by natural immunity and infection immunity.

Dr. Gentles: I have made the observation that it is very difficult to decide on examination of a hair whether the action is due to griseofulvin or to the formations of the hyphae during the formation of arthro-spores. Only with a very small percentage of hairs have I convinced myself that the action was probably due to the griseofulvin. I showed the best yesterday. Most hairs show the stoppage very abruptly and it is only in the odd hair where some of the hyphae or one hypha has grown downwards past this position of the junction of the stoppage that you get this malformation, which I believe is due to griseofulvin in the keratin.

I have also examined very carefully many nail specimens and skin scrapings from patients treated with griseofulvin; and I have not yet convinced myself that any of the changes I see in them, or any of the malformations of the hyphae, are due to griseofulvin. There are many hyphal forms—malformations, twistings and curlings—which occur in specimens from patients who have not received griseofulvin. One may, on some occasions, tend to believe that these forms are caused by the drug because one knows that the patient is being treated with it, but I have not found any effect which I can with certainty attribute to griseofulvin.

The Chairman: Thank you, Dr. Gentles. This seems to be something which will have to be put down for statistical analysis, doing it blind, I imagine.

Dr. C. J. La Touche: I would confirm the observation that the fluorescence tends to retreat to the tip in the hairs of animals not fed with griseofulvin. I have seen this in one control animal but I did not want to say anything about it because I wanted to get further evidence from other animals. I have been able to confirm this microscopically. One can see the hyphae bunched at the top of the hair and they do look abnormal. There was, however, no question of any effect of griseofulvin there. I am just waiting to see whether I can confirm this in other animals.

The Chairman: I think these comments of Dr. Gentles and Dr. La Touche are very salutary because most of us have looked over-critically probably at scrapings from patients who have been treated, and we assume far too readily that the changes we see are due to griseofulvin. Obviously, we have to look a lot more cautiously now at patients not receiving the drug.

It comes as a slight surprise to me that Dr. Gentles has come out so firmly. It is very refreshing to hear his comments about this, that he has failed to come to the conclusion that any of these changes in skin scrapings are definitely due to griseofulvin.

CORRELATION OF CLINICAL AND LABORATORY FINDINGS AND THE CRITERIA OF CURE

Brian F. Russell

It is the President's prerogative to wind up and summarise the discussions. I shall limit my remarks to the practical clinical aspects. First it would seem from my experience and from the discussions of the last two days, that the indications for treatment by griseofulvin are as follows: —

The indication is strong in Tinea capitis, all forms, except that due to microsporon canis, in which spontaneous resolution may occur without treatment. The indication is also strong in all forms of Favus, tinea unguium, Trichophyton rubrum infections and in Tokelau ringworm. The indications are moderate or relative in inflammatory zoophilic infections invading man. Dr. Whittle has indicated that their course may be shortened, and it seems that the cosmetic results are better with a lessened risk of keloid formation. The indications are doubtful or occasional in tinea pedis of the transient vesicular type, caused by Trichophyton interdigitale, Trichophyton mentagrophytes and Epidermophyton floccosum. No one has discussed this subject, but I think the general view is that these infections respond well to simple local treatment with potassium permanganate and fungicidal lotions and powders. It is only for completeness that I mention for this audience, that negative indications for treatment with griseofulvin exist in infections caused by non-susceptible organisms, such as Candida albicans, and in non-fungal conditions.

Clinical clearance usually precedes the microscopical evidence of mycological clearance, but the mycological condition may relapse even during treatment, in spite of apparent clinical clearance. It is clear that it is far too early to speak in terms of cure. If fungus persists on microscopic examination, it is essential to discover whether the fungus present is Candida Albicans or a pathogenic dermatophyte. It is only to be expected that cases will be reported of Candida replacing griseofulvin-sensitive fungi in diseased tissue. If a pathogenic dermatophyte persists, one must assume that one of the following causes is operative:

Non-co-operation of the patient.
Non-absorption from the gastro-intestinal tract.
Local inhibition of griseofulvin.
Inaccessibility of fungus in certain sites, or
The development of resistant strains.

When contemplating this problem, it is essential to remember that we are dealing with a fungistatic agent and not with a fungicide, and that clinical clearance is not necessarily synonymous with cure. The time factors vary so much in different situations. It is one thing to clip off rapidly growing hair, shampoo the scalp and clear up the infection in one fell swoop; it is another thing to eliminate infection and the risk of re-infection, when minute flakes of nail are becoming detached for the best part of a year. In war and in sport it is sometimes useful to put yourself in the place of the enemy. Here we have our fungus living in its Utopia. Shall I call it Keratopia. It comes under a suppressive regime. A mere existence can be maintained but not dynamic living. However escape is possible through the griseofulvin-ridden horny "curtain" to an outer sanctuary beyond the reach of the drug. Here the fungus can rise, like Phoenix from the ashes and resume its activities. Can pathogenic fungi go on living a precarious existence in the border country on the surface of keratin? Does this happen in the clefts? We have been told that fungus is resistant to griseofulvin in resting hairs. Can it be in the clefts, as Dr. David Williams has suggested, that the absence of hair follicles is important; or is the rate of keratinization possibly slower in this situation;

or is it all a matter of applying the principles of domestic dry-rot prevention to foot-rot in humans, that is, elimination of airless, damp and dark conditions? I can express the criteria of cure simply: "I do not know". Clearly the strict criteria of cure should be (1) Clinical clearance of all lesions, (2) Negative findings on direct microscopy, (3) Negative results of attempts at culture, and (4) (1), (2) and (3) above should all be found on at least three consecutive occasions. Should one add the old test of a round of golf in warm weather, wearing Wellington Boots?

We do not know enough about the host-parasite relationship; for example, there is great variability of clearance time between one patient and another. In our series the skin became normal in half of our patients after twelve weeks of treatment, but all of the remaining half were not clear for a further twenty four weeks. There are four important problems requiring clarification.

1. Zoophilic infections. These are essentially problems for co-operation between Dermatologist and Veterinary Surgeon. I would like to know about treated cattle. Do they relapse as easily as before on re-exposure?
2. Are anthropophilic fungi limited to the skin or are they ubiquitous in nature? If the former, this prevention of recurrence must depend on the elimination of reservoirs of infection in the immediate surroundings and contacts of the skin. If the latter, then attempts to prevent recurrence by attention to the environment would appear to be useless.
3. Is the immunological reaction of the patient modified by treatment with griseofulvin? Dr. Stevenson has presented evidence suggesting that it is, and this leads to the possibility that on re-exposure the intensity of recurrence may be modified.
4. A useful comparison can be made with staphylococcal infections, in which there are carriers and non-carriers, and in which clinical clearance may be followed by recurrence because of re-infection from without, or from a reservoir of infection somewhere on the individual concerned.

With reference to Trichophyton rubrum, what do we know of the existence of carriers of infection in toe nails, toe webs and other sites, and how great is the risk of re-infection from footwear, bathroom furniture or the general environment? I believe that in future we must pay as much attention to the environment, both animate and inanimate, as to the patient's skin, if we are to obtain a better understanding of our problems.

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LISLE STREET, LEICESTER SQUARE
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